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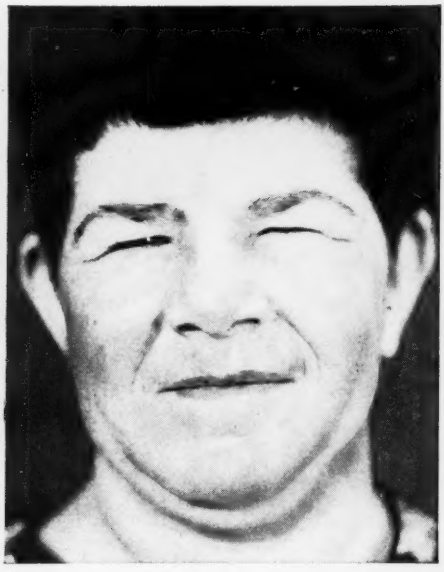

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THE ROLE OF THE HYPOTHALAMUS IN THE CONTROL OF THYROID FUNCTION*

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FOR many years it has been suspected that the nervous system exerts a controlling influence on thyroid function. It has been difficult, however, to obtain unequivocal evidence of this influence. Denervation or transplantation of the thyroid gland does not seem to interfere significantly with its function; it has therefore been assumed that there is no direct secretomotor innervation. Since hypophysectomy will reduce thyroid function to a minimum and, conversely, the administration of pituitary extracts will increase thyroid activity, any neural control of the thyroid gland would in all probability be mediated through the pituitary gland.

Several investigations have been made in the past of a possible hypothalamic influence on thyroid metabolism, but the criteria used were generally so inadequate that definite conclusions were not possible. These earlier investigations have been reviewed by Harris (1) and Ingram (2), hence an extensive discussion will not be undertaken here. A few experiments may be briefly noted, however.

Grafe and Grünthal (3) found that a fall in oxygen consumption fre-

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quently followed the injection of a necrotizing solution into the hypothalamus of dogs. Some of these animals had extensive lesions of the diencephalon at necropsy; in others none could be found, indicating that this effect may have been nonspecific. Uotila (4) noted that transection of the infundibular stalk in rats prevented an increase in thyroid cell height when the animals were exposed to cold, although there was no difference in thyroid weight between the experimental and control groups. The number of animals used was so small and the differences in cell height so slight, however, that the significance of the results has been questioned.

Westman and Jacobsohn found histologic evidence of thyroid hypofunction in both the rat (5) and rabbit (6) after stalk-section. Brolin (7), in addition, noted that in the rat this operation prevented post-thyroidectomy hypertrophy and vacuolization of the pituitary basophils. More recently Barrnett and Greep (8) have found that stalk-section greatly diminished the increase in thyroid weight following thiouracil feeding in the rat.

Although many investigations have been made in which accurate hypothalamic lesions have been produced with the Horsley-Clarke stereotaxic instrument, none has apparently been particularly concerned with thyroid function. Lesions in the hypothalamus which would produce changes in the thyrotropic secretion of the pituitary in the absence of any concomitant gross pathologic changes, such as infarcts, in the pituitary gland would clearly indicate central nervous control of hypophysial secretion. With the greatly improved methods of assessing thyroid function now available, it was initially decided to reinvestigate this problem by observing the change in thyroid reactions following destruction of various diencephalic areas.

Electrolytic lesions in the hypothalamus were produced with a machine patterned after Krieg's (9) modification of the Horsley-Clarke stereotaxic instrument. As experimental animals, 230–300 Gm. male and female albino rats of several different strains were utilized. In order to provide a maximal stimulus for the production of thyrotropin the animals were fed a diet of 0.03 per cent propylthiouracil for ten days, after allowing from one to three weeks for recovery from the operation. The animals were then killed and the thyroids weighed. Since previous work (10, 11) had shown that the thyroid/serum iodide ratio was the most sensitive index of endogenous thyrotropic activity in the rat, this was also determined.

Preliminary experiments had indicated that certain lesions in the anterior hypothalamus prevented the thyroid growth response to thiouracil, whereas lesions in the posterior hypothalamus, including the mammillary bodies, had no appreciable effect. Accordingly, a series of animals received bilateral hypothalamic lesions, 1 mm. from the midline and 0.5 to 1.0 mm.

from the base of the skull. These lesions were placed in a cephalocaudal plane from the ventromedian to the suprachiasmatic nucleus.

Of the 9 animals so prepared, none had an enlarged thyroid at the end of the ten days of thiouracil administration, and only 1 showed mild hyperplasia microscopically (12). The average thyroid weight of the hypothalamic animals was 5.9 mg. per 100 Gm. of body weight, whereas that of 12 similarly thiouracil-treated controls was 12.8 mg. The average thyroid weight of untreated animals in the laboratory was 7–8 mg. per 100 Gm. of body weight, so that the rats with hypothalamic lesions had thyroid

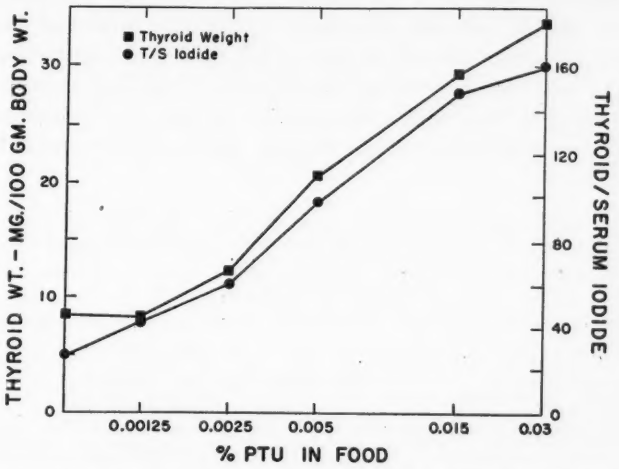

FIG. 1. Semilogarithmic plot of thyroid weight and thyroid/serum iodide ratio of 150-Gm. male Sprague-Dawley rats fed graded doses of propylthiouracil for fourteen days. Ten animals were used with each level of propylthiouracil. Thyroid/serum iodide ratio was determined with I^{131}, as previously described (11).

glands actually somewhat smaller than normal. Microscopically, the thyroid epithelium was either flat or low cuboidal except for the aforementioned one animal with mild hyperplasia. The pituitary gland was damaged in only 2 of the hypothalamically-treated animals; one had a large infarct affecting both lobes of the anterior pituitary, and the other had a small infarct in the left lobe. These observations have apparently been confirmed by Bogdanove and Halmi (13).

The most surprising finding was the marked increase in thyroid/serum iodide ratio in the absence of anatomic evidence of increased thyroid activity. The ratio is 25 in the normal rat (10). This was increased in the thiouracil-treated control animals by a factor of 10, averaging 264. Excluding the 2 animals with pituitary infarcts and the 1 with moderate

microscopic hyperplasia, the average in the hypothalamically-treated animals was 232, likewise a tenfold increase. If these 3 animals were included in the group with hypothalamic lesions, the figure was 214.

These findings raised the question of whether the apparent dichotomy between function and growth was due to the thyroid/serum iodide ratio having a lower threshold than the growth response of the cells to thyrotropin, or whether two different thyrotropic factors were involved. In order to clarify this point, propylthiouracil in graded concentrations from 0.00125 to 0.03 per cent was added to a synthetic diet and fed to 150-Gm. male Sprague-Dawley rats for fourteen days. It was thought that this experiment would provide gradually increasing levels of endogenous thyrotropin secretion. It can be seen in Figure 1 that the increase in thyroid weight and the increase in the thyroid/serum iodide ratio were remarkably parallel. This would indicate that in the rat the response threshold of the thyroidal iodide concentration was not lower than that of the weight response.

TABLE 1. MALE SPRAGUE-DAWLEY RATS (100 GM.) FED 0.06 PER CENT PROPYLTHIOURACIL FOR TEN DAYS AND GIVEN GRADED DOSES OF THYROXIN DAILY BY INTRAPERITONEAL INJECTION. THYROID/SERUM IODIDE RATIO DETERMINED AS PREVIOUSLY DESCRIBED (11)

No. of animals	Dose of thyroxin (μg.)	Body weight	Thyroid wt. (mg./100 Gm. body weight)	Thyroid/serum iodide ratio
12	2.5	133 ±10.7	9.4±1.4	70 ±21.3
12	3.5	117.3± 8.6	7.0±0.8	18.2± 7.6
12	5.0	126.2± 7.9	6.2±0.9	14.4± 7.0

In order to determine whether large doses of thiouracil, in the presence of small amounts of thyrotropin, might directly stimulate the thyroid gland to increased iodide concentration, 100-Gm. Sprague-Dawley male rats were fed a synthetic diet containing 0.06 per cent propylthiouracil for ten days. They were divided into three groups of 12 animals each and given 2.5, 3.5, and 5.0 micrograms, respectively, of racemic thyroxin daily by intraperitoneal injection during the experimental period. It was assumed that such treatment would allow three different levels of endogenous thyrotropin secretion near the normal range while maintaining maximally-inhibiting concentrations of propylthiouracil. According to Dempsey and Astwood (14), 3.5 micrograms of thyroxin is the daily requirement of a 100-Gm. rat.

It can be seen from Table 1 that when a dose of 2.5 micrograms of thyroxin is given there is a slight increase in both thyroid weight and the

thyroid/serum iodide. When the amount of thyroxin is increased to 3.5 micrograms, both thyroid weight and iodide concentration are maintained at normal levels. A 5-microgram dose causes both these factors to fall to levels slightly below normal. This indicates that the dichotomy observed in hypothalamic animals cannot be attributed to a direct effect of thiouracil on the ability of the thyroid gland to concentrate iodide.

An attempt has been made to define more precisely the thyrotropin-controlling area of the hypothalamus by mapping the entire diencephalon of the rat with small bilateral lesions, using a weaker current and smaller electrode than in the original investigation. To date 130 animals have been so investigated, but the results as yet are inconclusive, due to the difficulty of obtaining reproducible results with the smaller lesions and the necessity of determining the location with serial sections. The impression gained so far, however, is that the area involved is anterior to the ventromedian nucleus and lies along or near the ventral surface of the hypothalamus, possibly near the ventral extension of the supraoptico-hypophysial tract.

In a recent investigation conducted in collaboration with Dr. R. O. Scow and Dr. Clifford Grobstein, the effect of intraocular fetal and neonatal pituitary transplants on the target organs of hypophysectomized female mice has been studied (15). In such animals the pituitary is removed from any influence the hypothalamus may exert by direct contiguity. In implanted, hypophysectomized animals, the thyroid, adrenals, uterus, and ovaries lost weight to the same extent as in nonimplanted, hypophysectomized controls. In marked contrast to this, however, was the ability of the thyroid gland in the implanted mice to concentrate and bind iodine. Whereas the hypophysectomized controls had practically no accumulation of I^{131} three weeks after hypophysectomy, the implanted, hypophysectomized animals accumulated almost as much as the intact controls. When the mice were fed a thiouracil diet for three weeks, there was no increase in thyroid size in either the hypophysectomized or in the implanted, hypophysectomized animals, whereas the usual marked thyroid hypertrophy occurred in the intact controls. The increase in the thyroid/serum iodide ratio in the implanted animals was at least equal to that in the intact controls, but there was no increase above the usual hypophysectomy level in the nonimplanted, hypophysectomized mice.

It thus appears that a fractionation of the effects of the pituitary on thyroid function into two components can be demonstrated by the use of either bilateral, electrolytic hypothalamic lesions in the rat or of intraocular transplantation of the pituitary in hypophysectomized mice. A possible explanation of the accumulated data is that there are two thyrotropic principles secreted by the pituitary. One of these, arbitrarily named

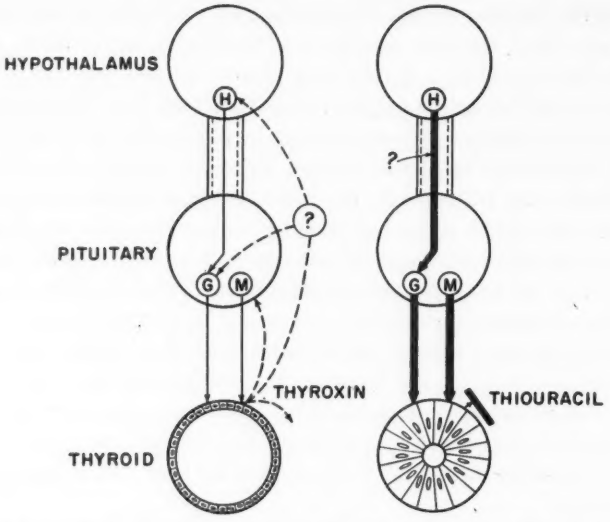

FIG. 2. Hypothetical diagrammatic representation of hypothalamo-hypophysial-thyroidal interrelation. The hypothalamus transmits an influence, H, through an intact pathway to the pituitary. This either stimulates or permits the secretion of the hypophysial thyrotropic "growth factor," G, which stimulates thyroid cell growth. An hypophysial "metabolic" thyrotropic factor, M, is not dependent upon the hypothalamus and stimulates thyroidal iodine metabolism including the secretion of thyroxin. Thyroxin, as well as stimulating general cellular metabolism, inhibits the secretion of both the growth and metabolic factors. The question mark indicates the uncertainty as to whether the growth factor is regulated by thyroxin at the hypothalamic or at the hypophysial level.

Thiouracil administration blocks the production of thyroxin, allowing maximal secretion of both the growth and metabolic factors. It is not known whether the hypothalamic influence is also increased in intensity under these circumstances.

the "metabolic factor," is independent of the hypothalamus and enables the thyroid to concentrate and bind iodine. This factor is apparently regulated, at least to a certain extent, by the level of circulating thyroid hormone, since the administration of antithyroid drugs will increase the iodide concentration in the thyroid and the administration of thyroxin will lower it. This regulation of pituitary secretion does not seem to be mediated directly through the nervous system.

A second factor, the "growth factor," again arbitrarily named, seems to regulate thyroid cell height and gross thyroid size. This principle apparently depends upon the integrity of a certain area of the hypothalamus and upon some manner of direct communication of this area with the anterior pituitary for maintenance. Suitably placed hypothalamic lesions, or intraocular transplantation of the pituitary, appear to interfere with

the secretion of this factor. The secretion of the "growth factor" also appears to be governed, at least in part, by the level of circulating thyroid hormone. Thiouracil administration will cause an increased growth of the thyroid gland, and thyroxin administration will cause the gland to atrophy. It is not clear whether the action of thyroxin is directly on the pituitary or on the hypothalamus. Although the growth factor requires the influence of the hypothalamus for the process of secretion, regulation of the rate may take place directly in the pituitary. Recent work by Jensen and Clark (16) demonstrating the concentration of radioactive thyroxin in the median eminence and pars nervosa indicates that regulation may occur in the hypothalamus.

It seems relatively certain that both growth and metabolic effects are mediated through hypophysial secretion, since hypophysectomy will cause marked diminution in the accumulation of iodine by the thyroid, as well as atrophy of the gland. Conversely, the administration of pituitary extracts will cause an increase in both thyroid size and iodine metabolism. The

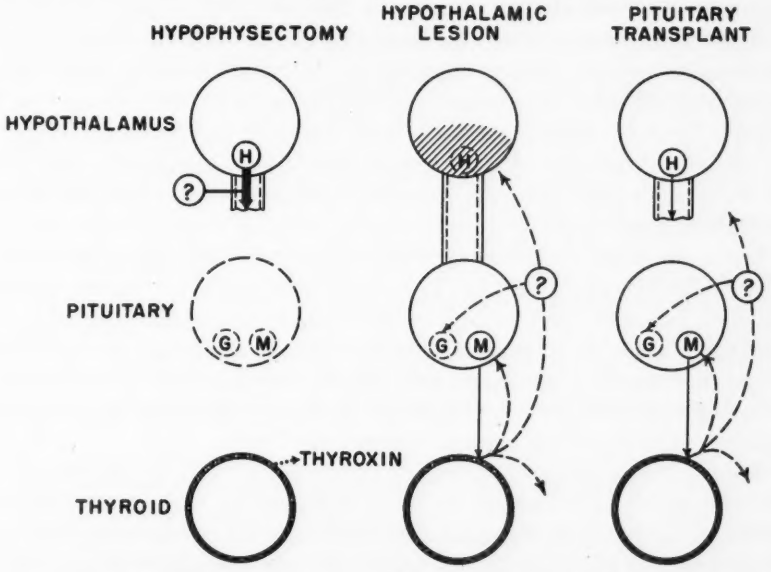

FIG. 3. With hypophysectomy, the secretion of both growth and metabolic factors is prevented. The thyroid atrophies and has a very low level of iodine metabolism. The minimal secretion of thyroxin may allow an increase in the hypothalamic influence, which has no effect since there is no pituitary gland upon which it can act.

Hypothalamic lesions or pituitary transplantation prevent any hypothalamic influence from reaching the pituitary. Consequently, only the metabolic thyrotropic factor is secreted. The thyroid gland atrophies but has a normal iodine metabolism and production of thyroxin.

fact that both effects seem to be present in approximately equal amounts in the currently available preparations of thyrotropin could mean that adequate fractionation has not been achieved by the relatively crude techniques so far employed. Of course, before the presence of two thyrotropic factors can be definitely established it will be necessary to achieve chemical separation, so that a clear-cut differentiation of the effects can be demonstrated following individual administration of the two principles.

It seems unlikely that the growth response of the thyroid gland is dependent upon the secretion of some endocrine gland other than the pituitary, in a manner similar to the dependence of the ovarian response to chorionic gonadotropin upon adequate adrenal secretion (17). The absence of thyroid hypertrophy following hypothalamic lesions is usually accompanied by normal estrous cycles in the female and normal adrenal weights. Conversely the thyroid usually hypertrophies normally in animals with persistent estrus or adrenal atrophy following hypothalamic lesions (18). The hypothalamic centers for the control of the various tropic pituitary secretions would thus seem to be discrete.

It is not known whether the lesions in the hypothalamus are actually destroying "centers" which regulate pituitary secretion or whether the areas involved merely transmit tracts from higher centers. In view of the apparently great importance of the hypothalamus as a regulator of homeostasis, however, it would not be surprising if it is eventually found that the influence comes from the hypothalamus itself and is not transmitted from other areas.

From the data available, it is not clear just what the mechanism of hypothalamic control may be. This may be mediated by neural or neurohumoral impulses, or possibly by some other transmission system. The inability of most investigators to demonstrate a significant nervous supply of the pars anterior, combined with the results of recent investigations of neurohumoral control (19, 20), would indicate that neurohumoral transmission, at least for the present, seems more likely.

That a dual control of endocrine function may not be peculiar to the thyroid gland is indicated by investigations of the adrenal-pituitary relationship. It seems clear that intraocular pituitary transplants in hypophysectomized rats (21–23) or mice (15) are incapable of maintaining adrenal size. Nevertheless, the pituitary is capable of stimulating adrenal function, as evidenced by a fall in adrenal ascorbic acid (21) or in the level of circulating esoinophils (23), in response to stress. This may mean that, as has been suggested (24), there is a weight maintenance factor and an ascorbic acid factor, rather than a single corticotropin.

SUMMARY

A dichotomy between the ability of the pituitary gland to maintain thyroid size and to stimulate thyroidal accumulation of iodine has been demonstrated. When the pituitary is not in direct contiguity with the hypothalamus or when certain areas of the anterior hypothalamus are destroyed by electrolytic lesions, the hypophysis is not able to stimulate the growth of the thyroid cells but is nevertheless able to maintain an approximately normal thyroidal iodine metabolism. This indicates that the pituitary may secrete two thyrotropic factors, arbitrarily called the "growth factor" and the "metabolic factor." The growth factor is apparently dependent upon the direct contiguity of the hypothalamus for its secretion. The metabolic factor is not significantly dependent upon the hypothalamus. Both factors seem to be regulated, at least in part, by the level of circulating thyroid hormone.

It is not known by what means the hypothalamus exerts its control over the growth factor. This control may be either neural or neurohumoral, the latter appearing more likely.

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RADIOACTIVE THYROTROPIC HORMONE PREPARATIONS*†

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RECENT investigations have established some of the physiologic reactions of the thyrotropic hormone and have raised other questions as to its role in health and disease. Studies made with radioactive iodine indicate that the thyrotropic hormone will promote the release and uptake of thyroid iodine. Leblond and co-workers (1, 2, 3) have demonstrated that the thyroid-stimulating hormone (TSH) will promote the collection of radioiodine by the thyroid, and conversely, hypophysectomized rats will show less accumulation of iodine by the thyroid than intact control rats. The first effect of TSH on iodine metabolism in the thyroid would appear to be the purging of the thyroid of its stored iodine (4). This loss of iodine would appear to precede the hypertrophy of the thyroid cell which occurs when TSH is administered (5). De Robertis and his associates (6–11) have suggested that TSH activates to some extent a proteolytic enzyme present in the thyroid colloid.

Although the mode of action of TSH on the thyroid cells is unknown, Rawson and co-workers (12) have suggested that in exerting its influence on the thyroid, the thyroid-stimulating hormone is inactivated. The only

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† Part of the dissertation of Martin Sonenberg in the Department of Physiological Sciences submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at New York University.

This work was partly carried out during a tenure of a fellowship in Cancer Research of the American Cancer Society and supported in part by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. This study also received joint support from the Office of Naval Research and the Atomic Energy Commission under Contract No. N8 onr–668.

This investigation was supported (in part) by a research grant (#G-3225) from the National Institutes of Health, Public Health Service.

** Damon Runyon Senior Clinical Research Fellow.

‡ Special Public Health Research Fellow of the National Cancer Institute.

other tissues which caused any inactivation of TSH *in vitro* were the thymus and lymph nodes.

The involvement of the thyroid-stimulating hormone in disease has not been so well delineated. It has been observed (5) that the urine of patients with untreated Graves' disease contains no active TSH, whereas after treatment of the patients with iodides, thyrotropic substances have been observed in the urine. Thyroid tissue of patients with Graves' disease, with *in vitro* techniques, was found to inactivate more TSH than normal thyroid tissue (13). D'Angelo and his associates (14) have attempted to correlate the TSH of human sera of normal and endocrinopathic individuals.

Extrathyroidal sites of action of the thyroid-stimulating hormone have been suggested. In the absence of the thyroid, histologic (15–17) and chemical (18) changes in the retro-orbital space have been demonstrated after the administration of thyrotropic hormone preparations. Structural alterations in skeletal muscle (16) have also been noted when experimental animals were treated with TSH.

The distribution and rate of metabolism of TSH in health and disease would also be of considerable interest. The recent success in the labeling of anterior pituitary hormone preparations (19–26) suggested that some insight into these problems might be gained by employing radioactively labeled thyrotropic hormone preparations in the experimental animal.

The selective accumulation of radioactivity in the adrenal cortex (19, 20, 21, 24) after administering I^{131}-labeled ACTH preparations or in the corpora lutea after radioactive prolactin preparations (22–24) is evidence that the histologic sites of action of such preparations may be determined with this technique. Interesting information has also been obtained from the rate of breakdown and disappearance of such labeled preparations from blood and organs, as well as the characteristic distribution of radioactivity.

Radioactive iodine as a label, as was previously employed with the other anterior pituitary hormone preparations, was not used because the thyroid accumulates this element normally. Since a derivative of a protein may be prepared by coupling with various diazotized compounds (27), it was decided to couple the thyrotropic hormone preparations with diazobenzene sulfonic acid, labeled with S^{35}. This approach has been used (28) successfully in the labeling of insulin with radioactive diazotized *p*-iodo aniline. The absorption of such radioactive insulin preparations from the site of injection was then ascertained. Trace amounts of labeling reagent were employed, inasmuch as the average number of substituent groups introduced will influence the degree to which biologic properties are altered. This reagent has been used to label antibodies (29) and antigens (30, 31).

METHODS

A. *Chemical*

Carrier-free S^{35}-labeled sulfanilic acid was prepared by a modification of the bake process and purified by paper chromatography.

Fifty microcuries of carrier-free S^{35} sulfanilic acid were evaporated to dryness on a boiling water bath under a stream of nitrogen. All subsequent steps were carried out at 4° C. To this material were added in turn, 10 micrograms of sulfanilic acid (in 0.1 cc.), 0.1 cc. of 0.2M sodium nitrite and slowly, in dropwise fashion, 0.1 cc. of 0.18 N sulfuric acid. The excess nitrous acid was destroyed with 0.1 cc. of 1M ammonium sulfamate. This entire solution was added to 3.3 mg. of a thyrotropic hormone preparation (Parke, Davis 099802)[1] dissolved in 0.1 cc. of a 1 molar solution of sodium carbonate-sodium bicarbonate, pH 8.8. This was allowed to remain in the cold (4° C.) for one hour and then transferred to a Visking dialysis tube. The preparation was dialysed in the cold (4° C.) with motor-driven stirring against four 17-liter changes of isotonic saline over two days. (There were only traces of radioactive sulfur in the third dialysate and no radioactivity could be determined in the fourth dialysate.) The nature of the radioactive components in the dialysate was not determined. The contents were brought to a concentration of approximately 300 micrograms of thyrotropic hormone preparation per cc. for injection purposes. Bovine serum albumin preparations were labeled in a similar fashion with S^{35}-labeled diazobenzene sulfonic acid.

In order to determine the extent of labeling, a portion of the coupled protein in the above reaction mixture before dialysis was mixed with an equal volume of 10 per cent trichloracetic acid. The mixture was centrifuged, the supernatant liquid removed, and the precipitate washed twice with 5 per cent CCl_3COOH. Counts were made in the total precipitate fraction and in the supernatant fraction in order to determine the percentage of radioactivity on the protein. (About 40 per cent of the radioactivity was found in the trichloracetic acid precipitate.)

B. *Biologic*

The results with 110 one-day-old male chicks are reported in this study. For the assay of biologic activity, 10 one-day-old, sex-linked male chicks were included in each group and six groups were employed. Each chick received 0.5 mg. of the labeled or unlabeled thyrotropic hormone preparation subcutaneously over a three-day period. Each daily injection consisted of the hormone preparation in 1 cc. of saline. One group of chicks received no injection. The animals were sacrificed one day after the last injection, at which time the thyroids were removed, dissected free of adjacent tissue and weighed.

In the localization studies the labeled hormone preparation was injected intravenously into a wing vein. At various periods of time after the injection, the chicks were sacrificed by exsanguination under ether anesthesia and portions of representative organs (thyroid, adrenal, gonads, muscle, thymus, liver, blood, orbital contents) were immediately removed, dissected free of adjacent tissue and placed in specimen vials containing 1 cc. of 0.1 N sodium hydroxide.

This procedure was repeated with control animals, which received bovine serum al-

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[1] We should like to thank Parke, Davis and Company for making this preparation available to us.

This pituitary powder was prepared by a modification of the method of Fraenkel-Conrat (32). Biologic assay by the chick thyroid weight technique revealed 6–8 Junkmann-Schoeller units per milligram.

bumin coupled with S^{35}-labeled diazobenzene sulfonic acid. S^{35}-labeled sulfanilic acid or S^{35}-labeled sulfate was administered to other control chicks.

The tissues were digested overnight at 90–100° C. The contents were transferred to cup planchettes and dried under an infrared lamp. The radioactivity in the samples was determined by an end-window Geiger-Mueller counter. Corrections for self-absorption were made on the basis of a previously determined curve obtained when samples of known thickness and intensity of radiation were assayed for radioactivity. The results have been expressed as the fraction of injected material per gram of tissue, as well as the organ/blood ratio of S^{35} concentration.

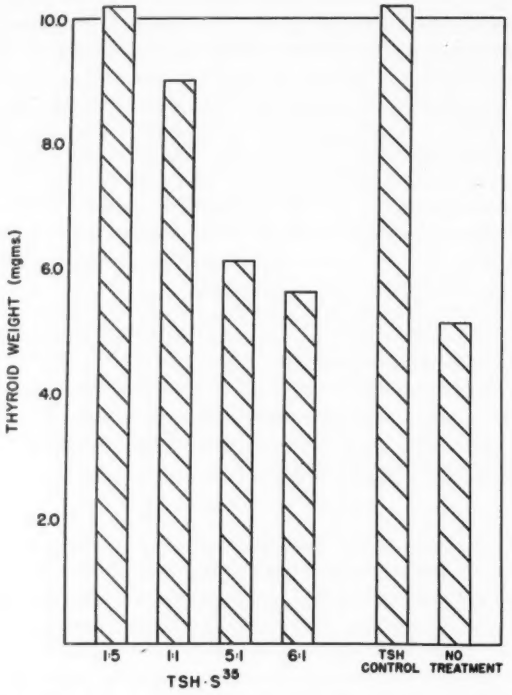

FIG. 1. Biologic activity of various thyrotropic hormone preparations as assayed by the thyroid weight of chicks. The ratios 1:5, 1:1, 5:1, and 6:1 represent the molecular ratios of S^{35}-labeled substituent group to protein. TSH control refers to the assay of an unlabeled thyrotropic hormone preparation.

RESULTS

The biologic activity of our labeled thyrotropic hormone preparations as measured by their ability to increase the thyroid weight of the chick was gradually lost as increasing numbers of substituent groups were introduced (Fig. 1). When these were labeled with one diazotized group per five protein molecules, the preparations demonstrated as much biologic

activity as an unlabeled thyrotropic hormone preparation. When the molecular ratio approached 1 to 1, approximately 20 per cent of the biologic activity was lost. When there were five diazotized groups per protein molecule, only 20 per cent of the biologic activity remained. There was no demonstrable stimulation of the chick thyroid weight with preparations having a molecular ratio of 6 to 1.

Although this pituitary preparation was known to be heterogeneous, for simplicity in representing the molecular ratios of substituent group to protein, the assumption was made that we had a homogeneous protein of molecular weight 10,000 (33).

Following the intravenous administration of an S^{35}-labeled thyrotropic hormone preparation to the one-day or two-day-old male chick, radio-

TABLE 1. LOCALIZATION OF RADIOACTIVITY AT DIFFERENT TIMES AFTER THE ADMINISTRATION OF A LABELED THYROTROPIC HORMONE PREPARATION
(TSH S^{35} No. 15)

Time	Thyroid		Adrenal		Gonad		Thymus	
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood
5 min.	1.59	0.157	2.08	0.205	2.28	0.224	0.739	0.073
20 min.	1.38	0.310	1.90	0.426	2.13	0.480	0.390	0.081
90 min.	1.18	1.46	0.690	0.852	1.02	1.26	0.290	0.358
5 hrs.	0.97	2.02	—	—	0.69	1.44	0.400	0.835
24 hrs.	0.46	5.42	0.167	1.96	0.30	3.54	0.110	1.29

Time	Liver		Muscle		Eye		Blood
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.
5 min.	10.92	1.89	0.78	0.070	0.704	0.069	10.14
20 min.	15.80	3.54	0.99	0.222	0.519	0.116	4.46
90 min.	13.95	17.0	0.958	1.180	0.274	0.338	0.81
5 hrs.	15.50	32.3	0.466	0.972	0.282	0.587	0.48
24 hrs.	4.03	47.5	0.044	0.422	0.056	0.660	0.085

activity was detectable in most of the removed tissues (Table 1). The concentration of radioactivity fell gradually and at different rates in the thyroid, adrenal, eye, gonad, and thymus. In the muscle there was an increase in radioactivity to a maximum at forty-five minutes, after which the concentration fell gradually. The concentration of radioactivity in the liver rose to a maximum in about one and a half hours and remained at these high levels before falling slowly. The tissues with the greatest con-

centration of S^{35} were the liver, gonad, and thyroid. The orbital contents, adrenal, thymus, and muscle had lesser concentrations of radioactivity.

With similarly labeled bovine serum albumin, the concentration of radioactivity was lost from each organ from the peak at five minutes—the first time (Table 2) at which observations were made. The differences in concentration of S^{35} between the various tissues were less marked than when labeled thyrotropic hormone preparations were administered.

TABLE 2. LOCALIZATION OF RADIOACTIVITY AT DIFFERENT TIMES AFTER THE ADMINISTRATION OF A LABELED BOVINE SERUM ALBUMIN PREPARATION
(BSA S^{35} No. 12)

Time	Thyroid		Adrenal		Gonad		Thymus	
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood
5 min.	1.16	0.101	1.75	0.151	1.80	0.156	0.940	0.082
20 min.	1.02	0.121	1.92	0.228	2.40	0.286	0.470	0.056
90 min.	0.94	0.148	1.04	0.164	1.80	0.284	0.600	0.095
5 hrs.	0.66	0.135	1.20	0.246	1.40	0.287	0.460	0.095
24 hrs.	0.82	0.488	0.529	0.315	1.01	0.602	0.450	0.268

Time	Liver		Muscle		Eye		Blood
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.
5 min.	3.32	0.289	1.10	0.096	0.668	0.058	11.5
20 min.	2.20	0.262	1.49	0.178	0.531	0.063	8.40
90 min.	2.41	0.381	0.429	0.115	0.648	0.102	6.33
5 hrs.	2.57	0.528	1.028	0.211	0.549	0.113	4.87
24 hrs.	1.37	0.815	0.745	0.444	0.323	0.192	1.68

After the administration of S^{35}-labeled sulfate (Table 3), there was a rapid loss of radioactivity from all tissues except the muscle, in which there was an increase in concentration at twenty minutes. After reaching this maximum, the concentration fell to approximately the same levels observed with other tissues.

With S^{35}-labeled sulfanilic acid (Table 4), all organs showed a decrease in radioactivity from the first point observed at five minutes, except in the case of muscle, in which there was a peak concentration at twenty minutes. The concentration of radioactivity in the thymus showed a slower decline and there was even a slight increase at twenty-four hours.

When the concentration of radioactivity, expressed as the fraction of the administered dose which localized per gram of tissue, was related to the

TABLE 3. LOCALIZATION OF RADIOACTIVITY AT DIFFERENT TIMES AFTER THE ADMINISTRATION OF S^{35} LABELED SULFATE

Time	Thyroid		Adrenal		Gonad		Thymus	
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood
5 min.	1.28	0.338	1.23	0.325	1.71	0.452	1.36	0.359
20 min.	0.76	0.559	0.72	0.246	1.22	0.417	1.01	0.344
90 min.	0.60	0.280	0.93	0.435	1.12	0.523	0.92	0.430
5 hrs.	0.56	0.560	0.53	0.530	0.624	0.624	0.655	0.655
24 hrs.	0.44	1.42	0.46	1.48	0.430	1.38	0.740	2.38

Time	Liver		Muscle		Eye		Blood
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.
5 min.	0.920	0.243	2.32	0.612	0.781	0.206	3.79
20 min.	0.650	0.222	1.13	0.386	0.672	0.229	2.93
90 min.	0.810	0.379	0.680	0.317	0.729	0.341	2.14
5 hrs.	0.460	0.460	0.310	0.310	0.538	0.538	1.00
24 hrs.	0.430	1.38	0.117	0.378	0.375	1.21	0.31

TABLE 4. LOCALIZATION OF RADIOACTIVITY AT DIFFERENT TIMES AFTER THE ADMINISTRATION OF S^{35}-LABELED SULFANILIC ACID

Time	Thyroid		Adrenal		Gonad		Thymus	
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood
5 min.	0.980	0.267	1.12	0.305	1.35	0.368	0.820	0.223
20 min.	0.840	0.318	0.820	0.310	1.12	0.425	0.820	0.311
90 min.	0.250	0.247	0.430	0.420	0.500	0.490	0.430	0.425
5 hrs.	0.185	0.578	0.210	0.660	0.120	0.376	0.130	0.407
24 hrs.	0.120	1.01	0.070	0.590	0.080	0.670	0.082	0.690

Time	Liver		Muscle		Eye		Blood
	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.	Organ/ Blood	S^{35} Conc.
5 min.	1.40	0.381	2.19	0.593	0.829	0.246	3.67
20 min.	1.25	0.473	2.67	1.01	0.688	0.260	2.64
90 min.	0.450	0.440	0.662	0.656	0.321	0.318	1.01
5 hrs.	0.250	0.780	0.150	0.469	0.131	0.410	0.32
24 hrs.	0.090	0.830	0.180	0.155	0.052	0.437	0.119

concentration in blood similarly expressed, certain differences became apparent (Figs. 2–6). In the case of the thyroid, after the administration of an S^{35}-labeled TSH preparation, the thyroid/blood ratio of concentration of radioactivity was significantly greater than that for the control substances at one and a half, five, and twenty-four hours (Figs. 4–6) but not at five and at twenty minutes (Figs. 2 and 3). This general pattern was also followed when the gonad/blood and liver/blood ratios were studied. The thymic tissue and orbital contents did not appear to show these differ-

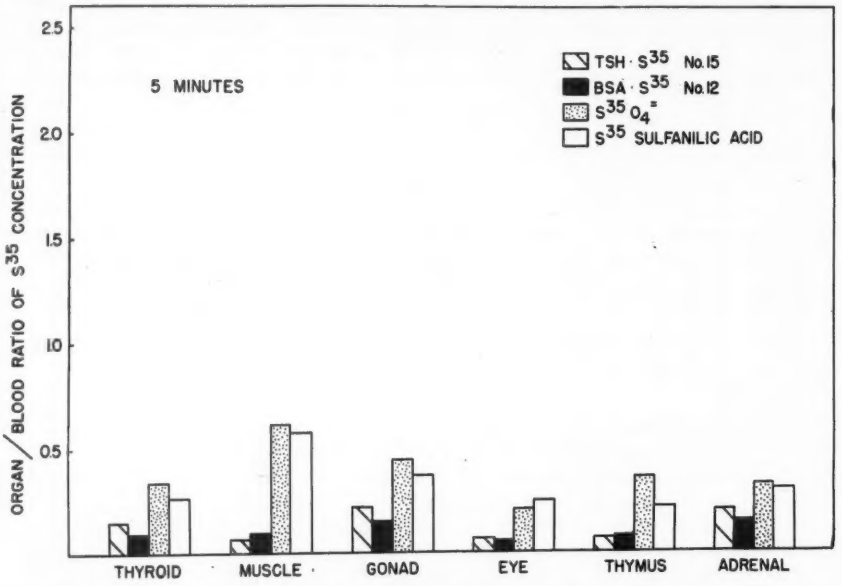

FIG. 2. The relationship of S^{35} concentration between various tissues and blood five minutes after the administration of different labeled substances.

ences between the labeled thyrotropic hormone preparation and the control substances. The muscle/blood ratio of S^{35} concentration was greater than 1, only at ninety minutes (Fig. 4) after the administration of the labeled pituitary preparation. The muscle/blood ratios were equivocally significant after the administration of labeled TSH preparations, as opposed to the ratios obtained after the injection of control substances.

DISCUSSION

At first examination the small degree of localization of radioactivity in the thyroid and gonads after administering supposedly biologically specific materials like thyrotropic and gonadotropic hormones is somewhat disconcerting. This may be accounted for in several ways. Firstly, our labeled

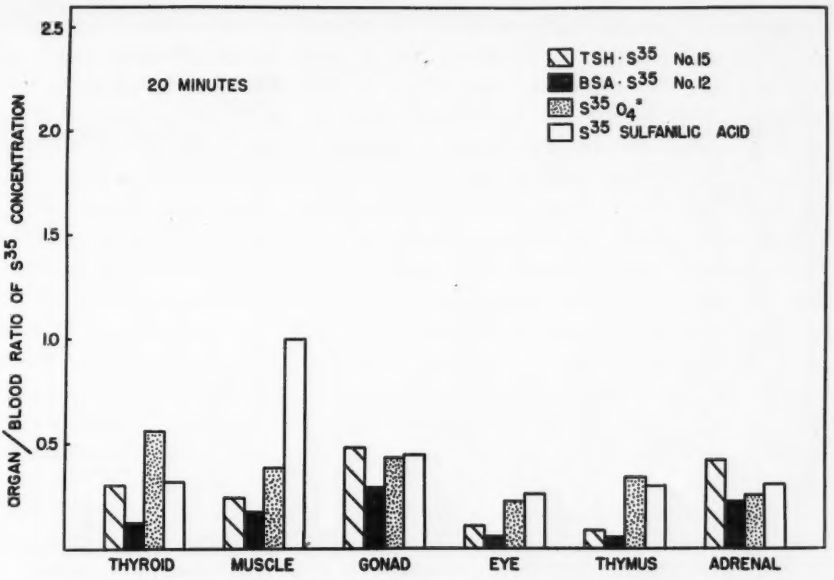

FIG. 3. The relationship of S^{35} concentration between various tissues and blood twenty minutes after the administration of different labeled substances.

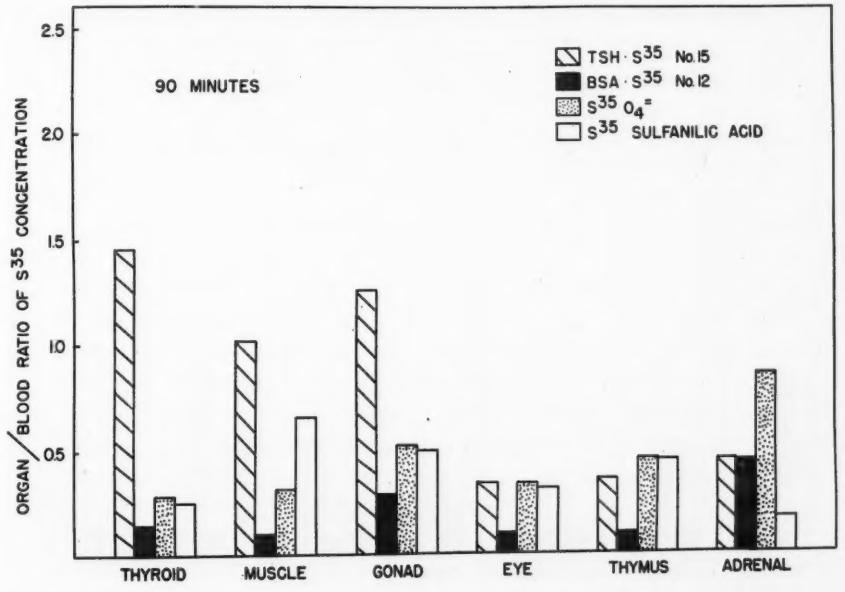

FIG. 4. The relationship of S^{35} concentration between various tissues and blood ninety minutes after the administration of different labeled substances.

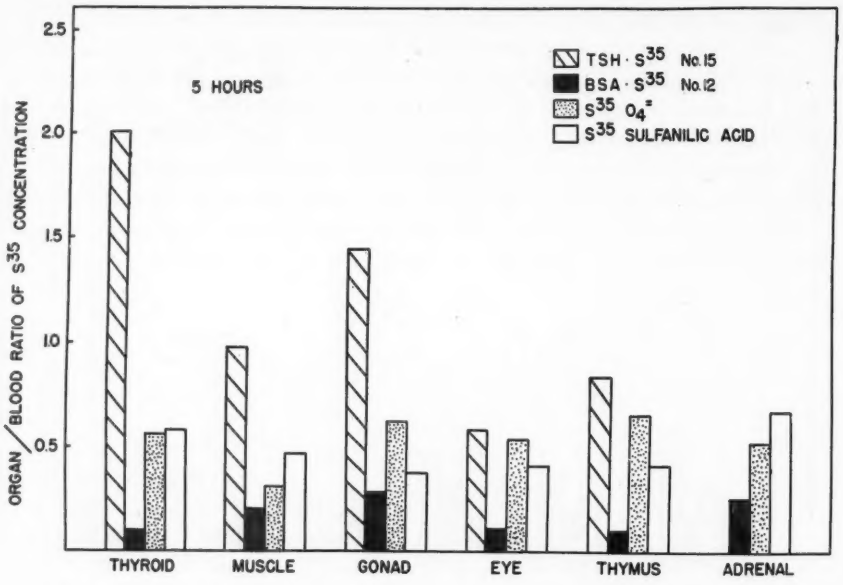

FIG. 5. The relationship of S^{35} concentration between various tissues and blood five hours after the administration of different labeled substances.

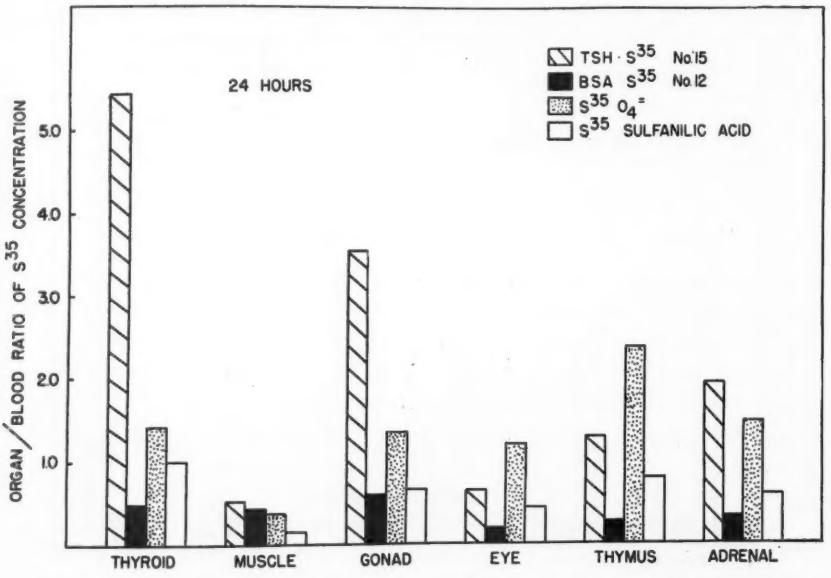

FIG. 6. The relationship of S^{35} concentration between various tissues and blood twenty-four hours after the administration of different labeled substances.

pituitary preparation was known to be inhomogeneous, as demonstrated in this laboratory by chromatography (Fig. 7). Biologic assay revealed the presence of large amounts of thyrotropic and gonadotropic activity in this preparation. Thus a large fraction of the radioactively labeled thyrotropic hormone preparation may well represent nonspecific protein or another biologically active protein, the distribution of which would completely mask that of the biologically specific smaller fractions. The high concentration of radioactivity in the liver after administering a radioactively labeled TSH preparation may be in part the result of the action of the reticulo-endothelial system on nonspecific protein. However, the localization of some more specific component in the liver has not been excluded. Secondly, the small blood flow of the thyroid and gonads with reference to the rest of the body would limit the amount of TSH preparation which could be cleared by the thyroid or gonads during the period of this experiment. The increase in the thyroid/blood and gonad/blood ratios of S^{35} concentration at the later periods of time suggests that the thyroid and gonads are able to retain from the substances presented to them, a greater proportion of the specific material than of the nonspecific material. The increased thyroid/blood and gonad/blood ratios at the later periods of time may also reflect a difference in the rate of loss of radioactivity in these two tissues and blood. The radioactivity is lost from the blood at a more rapid rate. Thirdly, the TSH and gonadotropin may localize in and disappear rather quickly from the thyroid and gonads. This rate of disappearance, if proceeding at a more rapid rate than the rate of localization, would not permit any sizable accumulation of radioactivity in the thyroid gland or the gonads. This concept of the rapid action of thyrotropic hormone in the rat is supported by the observation that thirty minutes after the administration of a pituitary preparation with TSH activity, colloid droplets in the thyroid cells are apparent (8). A significant decrease in thyroid iodine is apparent within six hours (5) after a subcutaneous injection in the chick. Inasmuch as our TSH preparations were administered intravenously, we might anticipate a more rapid biologic effect. Fourthly, the possibility exists that some thyrotropin and gonadotropin may be destroyed in blood. This would reduce the amount of tropic factors for the target glands. Finally, it may well be that the dosage of TSH preparation used (300 micrograms per chick) was excessive. The introduction of doses more closely approximating the physiologic range might result in different localization patterns.

The greater radioactivity in the thyroid, gonads, and other organs is not accounted for on the basis of differences in blood volume, as is demonstrated with control studies. In the case of the thyroid, for example, in no instance was the thyroid/blood ratio of S^{35} concentration as great with

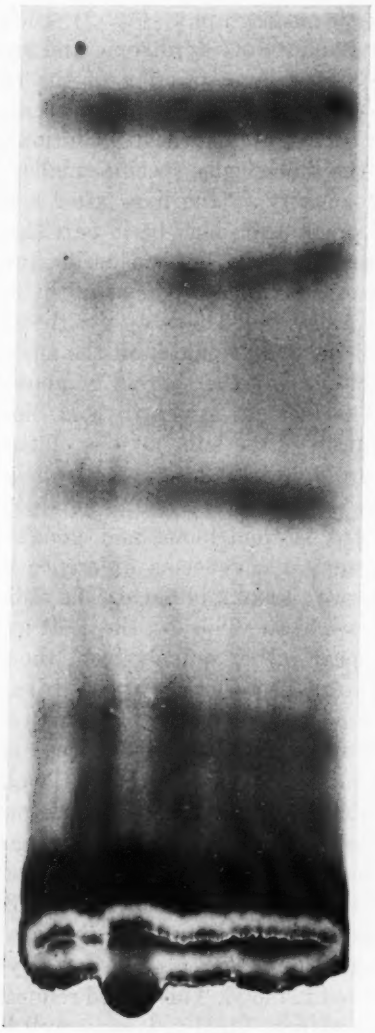

FIG. 7. Radioautograph of a paper chromatogram of a labeled thyrotropic hormone preparation (TSH S^{35} No. 15), developed on Whatman No. 1 paper in a solvent consisting of two parts by volume of normal propanol and one part by volume of $\frac{M}{10}$ phthalate buffer (pH 2.2).

S^{35}-labeled BSA, S^{35}-labeled sulfate, and S^{35}-labeled sulfanilic acid, as it was after the administration of a radioactively labeled TSH preparation. This was also the case for the gonads and liver, and to a lesser extent with the other organs, suggesting some specificity for the labeled TSH preparation. It is not likely that the disappearance of radioactivity can be related to the metabolism by the thyroid and gonads alone. The disappearance curves may well represent the phenomena of destruction of labeled thyrotropin, gonadotropin, and other biologically active factors by the thyroid, gonad, and other tissues, the metabolism of inorganic and organic sulfur compounds, and the metabolism of nonspecific protein coupled with diazobenzene sulfonic acid.

The determination of the biologic activity of a protein hormone which has had introduced different substituent groups is of interest. In the case of a heterogeneous protein preparation as was employed in these studies, the results have less significance, inasmuch as one may only describe the ratio of substituent group to protein as an average one. For a nonhomogeneous protein preparation, it is necessary to know the kinetics of labeling each constituent, the rates of which may vary widely (34). Although the biologic activity of our radioactively labeled TSH preparations decreased with larger amounts of radioactive substituent groups, it is difficult to be sure that the biologic activity manifested was due to a protein molecule labeled with more, less, or the average number of substituent groups. It is difficult to predict, *a priori*, the behavior of thyrotropin or gonadotropin in this regard. Insulin coupled with six azo groups per protein molecule maintained its activity almost unchanged (35).

In the course of studies with labeled anterior pituitary preparations, certain patterns have evolved which suggest specificity of localized labeled components for a tissue (24). After the administration of a labeled preparation containing a specific factor, a greater concentration of radioactivity has been found in the target tissue as opposed to so-called nonspecific tissue. Secondly, it has been found that other labeled substances do not localize to the same extent in the target tissue as do the labeled specific factors. Thirdly, concentrations of radioactivity greater than blood have frequently been observed when radioactive specific components are administered. Fourthly, a direct correlation has been noted between the biologic assay and the localizing potential of a given preparation. Fifthly, radioactively labeled components which have localized in an organ are not lost from that tissue by perfusion, as is the case with nonspecific substances. Finally, the histologic site of action as demonstrated by radioautography has correlated well with other data which indicate the site of action.

It would appear that the specificity of our S^{35}-labeled pituitary prepara-

tion has been retained. The concentration of S^{35} is greater in the thyroid, gonads, and liver than in other tissues. Control substances do not manifest the same degree of localization of radioactivity. The organ/blood ratios for the thyroid, gonads, and liver are considerably greater than those observed with control substances, and are frequently greater than 1. An attempt is being made to satisfy the other criteria (24) of specificity of localized labeled components after the administration of a radioactively labeled pituitary preparation.

The localization of radioactivity in the thyroids and gonads of chicks receiving a radioactively labeled pituitary preparation rich in thyrotropic and gonadotropic activity might be expected. However, the possibility must be considered that the radioactivity found in the thyroid or gonads is from a constituent other than the thyroid-stimulating or gonad-stimulating factor.

Rawson and his associates (12) have reported that the exposure of TSH preparations to representative tissues of the rabbit resulted in inactivation of the TSH only with thyroid, thymus, and lymph nodes. Our studies do not reveal conclusive evidence of localization of radioactivity in the thymus after administering a radioactive TSH preparation. Histologic changes in muscle in thyroidectomized animals receiving TSH preparations (16) suggest some specific effect in this tissue. However, the effect of other substances in the pituitary preparations as well as nonspecific reactions has not been adequately defined. The results herein reported are mildly suggestive but not conclusive of any specific factor for muscle in a pituitary preparation with thyrotropic and gonadotropic activity. The finding of small concentrations of radioactivity in orbital tissue after administration of radioactive TSH preparations remains inconclusive. If the site of localization of an ophthalmotropic factor were but a small fraction of all the tissue removed from the eye socket, this concentration would be masked by the large amount of nonspecific tissue present. If a factor in a pituitary preparation with TSH activity is specific for some component in the orbital tissue, it may be present in exceedingly small amounts. Jefferies (36, 37, 38) has suggested that such an ophthalmotropic factor is distinct from the thyrotropic factor. It may well be that, unlike the results with thyrotropic and gonadotropic components, labeling by coupling with diazobenzene sulfonic acid may destroy the localizing potential of such a component. The possibility must also be entertained that there is no specific component in a pituitary preparation for any orbital tissue.

It is interesting to note the moderately long biologic half-life of the radioactivity in the thyroid and gonads (Fig. 8). This was approximately eighteen hours. It was significantly different from the half time of disappearance (five and half minutes) of radioactivity from the adrenal of the

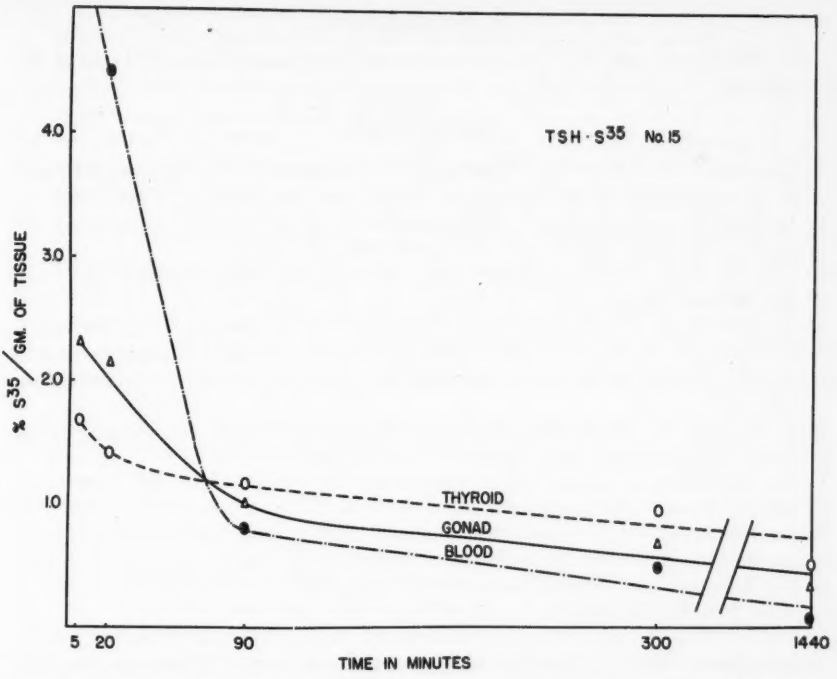

FIG. 8. Relationship of time and concentration of radioactivity in the thyroid, gonads and blood after the administration of a labeled thyrotropic hormone preparation.

rat after administering a radioactive ACTH preparation (19, 22). In plasma, ACTH as determined by adrenal ascorbic acid depletion, had a biologic half-life of five and a half minutes (39). In the ovary of the rat, the half-time of disappearance of radioactivity was approximately ninety minutes when a radioactive prolactin preparation was administered (23, 24). When I^{131}-labeled luteinizing hormone preparations were administered to the rat, the radioactivity in the ovary fell, with a half-life of eighty-five minutes (24).

SUMMARY

A pituitary preparation with thyrotropic and gonadotropic activity has been coupled with S^{35}-labeled diazobenzene sulfonic acid.

The biologic activity of labeled pituitary hormone preparations with reference to thyrotropin has been found to decrease with an increasing number of substituent groups on the protein.

In the localization studies, significant concentrations of radioactivity have been found in the thyroid, gonads, and liver after administering labeled TSH preparations to the male chick.

Acknowledgments

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THE MECHANISM RESPONSIBLE FOR THE ALTERED BLOOD CHOLESTEROL CONTENT IN DERANGED THYROID STATES*†

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I. INTRODUCTION

ALTHOUGH it is almost thirty years since abnormalities of blood cholesterol content were observed to follow deranged thyroid activity (1), the mechanism responsible for these changes remains unknown. Moreover, the obscurity surrounding this interesting thyroid-blood cholesterol relationship has persisted, despite the clinical recognition (2) of a possible association between the hypercholesteremia of the hypothyroid state and the premature atherosclerosis believed by many to occur in this disease.

It appeared important to investigate some of the underlying processes concerned with the altered blood cholesterol level in both the hyperthyroid and hypothyroid states. By using various new techniques we have been able to discover that the underlying fault in cholesterol metabolism in the hyperthyroid state is one in which an increase occurs, not only in the rate of manufacture, but also in the rate of excretion and destruction of cholesterol. We have also found that, conversely, the same processes are slowed in the hypothyroid state.

II. EXPERIMENTAL PROCEDURES

The experimental data to be described were obtained from studies in rats (Long-Evans strain) rendered hyperthyroid or hypothyroid by the incorporation of either powdered thyroid substance or thiouracil, respectively, into the diet. The exact details by which these states were induced have been described in previous studies (3, 4). All chemical determinations of cholesterol and of cholate were made according to previously published methods (5–7). The determination of the radioactivity of cholesterol

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labeled with tritium was made according to the technique of Biggs and associates (8).

III. RELATIONSHIP BETWEEN THYROID ACTIVITY AND PLASMA CONTENT OF CHOLESTEROL

The plasma cholesterol level rises in the hypothyroid state and falls in the hyperthyroid state (9). This inverse relationship is found in man (9, 10) as well as in various animals (11–14), although it appears that no close or immediate relationship exists between blood cholesterol changes and (a) basal metabolic rate, (b) blood protein-bound iodine content, or (c) changes in other blood lipid fractions (9, 10, 15).

TABLE 1. THE CHANGES IN AVERAGE PLASMA CHOLESTEROL CONCENTRATION AFTER PRODUCTION OF HYPO- AND HYPERTHYROIDISM

Type of rat	No. of rats	Plasma cholesterol concentration (mgm./100 cc.)					
		Before medication			After medication		
		Total	Free	Ester	Total	Free	Ester
Control	29	54 (S.E.$_m$ ±1.8)*	5.9	48.1	54 (S.E.$_m$ ±2.4)	7.2	46.8
Hypothyroid	45	47.3 (S.E.$_m$ ±1.2)	4.5	42.8	67.2 (S.E.$_m$ ±1.6)	13	54.2
Hyperthyroid	41	50.4 (S.E.$_m$ ±1.6)	7	43.4	40 (S.E.$_m$ ±1.3)	6.6	33.4

* S.E.$_m$ = standard error of the mean.

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In the present studies the plasma cholesterol content was decreased in the hyperthyroid rat (Table 1), as others previously have reported (14). Correspondingly, the plasma cholesterol content of the hypothyroid rat was increased.

IV. EFFECT OF VARIATIONS IN DIETARY CHOLESTEROL ON THE PLASMA CHOLESTEROL CONTENT IN HYPERTHYROID AND IN HYPOTHYROID STATES

Although there has been evidence (12b, 14) that ingestion of excess cholesterol exaggerates the hypercholesteremia found in the hypothyroid state, restriction of dietary cholesterol does not appear (10e) to affect the plasma level of cholesterol either in the hyperthyroid or in the hypothyroid state. Moreover, possible changes in intestinal absorption of

cholesterol have not been found (10e, 16, 17) to account for the blood cholesterol changes in hyperthyroid and hypothyroid states.

Our own studies have confirmed the foregoing observations, in that feeding a diet completely devoid of sterols to 6 hypothyroid rats led to an average plasma cholesterol value of 117 mg. per 100 cc., which was even higher than the average level of 80 mg. per 100 cc. in 6 control hypothyroid rats on a normal diet (18). Furthermore, as the later experiments will indicate, changes in the rates of synthesis and elimination of endogenous cholesterol are so profound that no other changes appear necessary to explain the observed changes in blood cholesterol concentration.

V. DISTRIBUTION OF CHOLESTEROL IN HYPERTHYROID AND IN HYPOTHYROID STATES

The total body content of cholesterol of hyperthyroid animals has been reported as increased by some (16), whereas others have found it either unchanged (19) or decreased (20).

TABLE 2. VISCERAL CHOLESTEROL CONTENT IN HYPO- AND HYPERTHYROID RATS

Type of rat	No. of rats	Total cholesterol in viscera (mg.)
Normal	5	65.4* (52–73)†
Hypothyroid	5	68.0 (53–70)
Hyperthyroid	5	71.2 (69–84)

* Median.
† Range.

In order to determine whether the body content of cholesterol changes in the hyperthyroid and in the hypothyroid rat, the following experiment was carried out: 5 hyperthyroid, 5 hypothyroid, and 5 control rats were killed, the viscera hydrolyzed, and their nonsaponifiable material extracted with ether. The visceral cholesterol was precipitated with digitonin and its concentration then ascertained (7, 21). In Table 2 it can be noted that the total content of visceral cholesterol was slightly increased in both the hyperthyroid and the hypothyroid rats. Therefore, it seems unlikely that either excess or deficiency of thyroid hormone altered the plasma level of cholesterol by leading to its redistribution between the blood and the fixed body tissues.

VI. RATE OF SYNTHESIS OF CHOLESTEROL IN HYPERTHYROID AND IN HYPOTHYROID STATES

Several observations (22, 23) have suggested that the rate of synthesis of lipids may be increased in hyperthyroidism. It therefore was considered

important by us to study the rate of synthesis of cholesterol in the hypothyroid and in the hyperthyroid rat. This was accomplished in several different ways.

First, the rate of synthesis of cholesterol by the liver was assayed by analysis of the cholesterol content of the bile in both types of rat (24). Such assay was found (25) to give a direct index of the rate of hepatic synthesis of cholesterol. The average concentration of cholesterol in the bile of 17 hyperthyroid rats (Table 3) was 42.1 mg. per 100 cc. and the total biliary cholesterol output was 5.6 mg. per day (3, 4). When these

TABLE 3. THE AVERAGE CONCENTRATION AND DAILY OUTPUT OF BILE CHOLESTEROL IN HYPO- AND HYPERTHYROID RATS

Type of rat	No. of rats	Bile		
		Volume (cc./24 hrs.)	Cholesterol	
			Concentration (mgm./100 cc.)	Content (mgm./24 hrs.)
Control	16	13.2	20.7 (S.E.$_m$ ±1.5)*	2.7 (S.E.$_m$ ±0.14)
Hypothyroid	17	12	9.8 (S.E.$_m$ ±0.8)	1.2 (S.E.$_m$ ±0.11)
Hyperthyroid	17	13.3	42.1 (S.E.$_m$ ±3.1)	5.6 (S.E.$_m$ ±0.56)

* S.E.$_m$ = standard error of the mean.

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data were compared with those obtained from 16 control rats (average biliary cholesterol concentration, 20.7 mg. per 100 cc.; average biliary output, 2.7 mg. per day) there could be no question but that there was a profound increase in the rate of cholesterol synthesis in the hyperthyroid rat. Conversely, the markedly reduced biliary concentration (9.8 mg. per 100 cc.) and output (1.2 mg. per day) of cholesterol found in 17 hypothyroid rats left little doubt that a marked slowing in the rate of cholesterol manufacture had occurred in these rats.

These changes in the rate of synthesis were demonstrated again by the differential rates of rise of the cholesterol level of the blood following biliary obstruction in the euthyroid, in the hyperthyroid, and in the hypothyroid rat (Table 4). Thus, when 21 hyperthyroid rats were submitted to biliary obstruction by ligation of the duct (4), the plasma cholesterol level rose much more quickly than in the 19 hypothyroid or in the 11 euthyroid rats.

TABLE 4. CHANGES IN AVERAGE PLASMA CHOLESTEROL CONCENTRATION AFTER BILE DUCT LIGATION IN HYPO- AND HYPERTHYROID RATS

Type of rat	No. of rats	Average plasma cholesterol concentration (mgm./100 cc.)						
		Before ligation			24 hours after ligation			Net increase after ligation
		Total	Free	Ester	Total	Free	Ester	(mgm./100 cc.) Total
Control	11	47.6	6.7	40.8	119 (S.E.$_m$ ± 3.2)*	49.3	69.7	72 (S.E.$_m$ ± 3.9)
Hypothyroid	19	65.6	12.3	53.3	115 (S.E.$_m$ ± 4.1)	46.2	68.8	49 (S.E.$_m$ ± 6.4)
Hyperthyroid	21	37.6	7.1	30.5	147 (S.E.$_m$ ± 8.7)	78.6	68.4	109 (S.E.$_m$ ± 9.0)

* S.E.$_m$ = standard error of the mean.

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Moreover, the faster rise observed in the hyperthyroid rat could not be due to any retardation in rate of elimination or destruction of blood cholesterol, as will be demonstrated later.

The foregoing changes in rate of cholesterol manufacture were confirmed again by means of "tracer" studies (7). Water "labeled" with tritium was injected intravenously into 5 hyperthyroid and 5 hypothyroid rats, as well as into 5 controls. Twenty-four hours later, during which time all food and water were withheld, the blood and viscera were hydrolyzed and the nonsaponifiable material was extracted with ether. The cholesterol was precipitated with digitonin and its radioactivity then determined (8). In Table 5 it can be seen that the rate of incorporation of tritium into the cholesterol molecule, as indicated by its specific activity, was markedly

TABLE 5. RATE OF CHOLESTEROL TURNOVER IN HYPER- AND HYPOTHYROID RATS

Type of rat	No. of rats	Cholesterol spec. activity (μc./Gm.)
Normal	5	0.7* (0.37–0.85)†
Hyperthyroid	5	1.6 (1.1 –3.5)
Hypothyroid	5	0.27 (0.2 –0.32)

* Median.

† Range.

increased in the hyperthyroid rats, and markedly decreased in the hypothyroid rats.

In summary, physiologic and tracer studies clearly have shown that the hyperthyroid state is associated with a considerably increased rate of hepatic synthesis of cholesterol, and that the converse is true in the hypothyroid state. Indeed, the increased rate of synthesis of cholesterol in the hyperthyroid state apparently utilizes acetate (26) at such a rapid rate that the amount of acetate available for other processes, such as acetylation of drugs, is inadequate (27).

VII. RATE OF ELIMINATION OF CHOLESTEROL IN HYPERTHYROID AND IN HYPOTHYROID STATES

The preceding results made it clear that if the rates of cholesterol synthesis are so markedly changed in hyperthyroid and in hypothyroid states, changes in the opposite direction probably occur in the excretory and/or destructive processes concerned with cholesterol. The following studies, therefore, were performed:

A. *Rate of disappearance of injected excess cholesterol from the blood of hyperthyroid and hypothyroid rats*

Temporary hypercholesteremia was produced in 7 hyperthyroid, 6 hypothyroid, and 5 control rats, by injecting plasma obtained from other rats in which a marked hypercholesteremia had been induced (28). The rate of disappearance of the excess plasma cholesterol then was observed by periodic determination of the plasma cholesterol concentration (4). Other studies with physiologic (25) and tracer (29) techniques have shown that such injected "physiologic" cholesterol is not eliminated in the bile, but

TABLE 6. RATE OF DISAPPEARANCE OF INJECTED CHOLESTEROL IN HYPO- AND HYPERTHYROID RATS*

Type of rat	No. of rats	Plasma cholesterol increments (mg./100 cc.)†				
		Immediately after injection	1 hour after injection	3 hours after injection	6 hours after injection	12 hours after injection
Control	5	114 (S.E. Mean: ±5.4)	113‡	69.6 (S.E. Mean: ±3.4)	33 (S.E. Mean: ±3.5)	9 (S.E. Mean: ±3.5)
Hyperthyroid	7	137 (S.E. Mean: ±7)	103 (S.E. Mean: ±6.5)	58.4 (S.E. Mean: ±8)	6 (S.E. Mean: ±4.1)	−18 (S.E. Mean: ±7.4)
Hypothyroid	6	127.6 (S.E. Mean: ±8.4)	118 (S.E. Mean: ±3.4)	90 (S.E. Mean: ±6.3)	51.5 (S.E. Mean: ±9)	10.8 (S.E. Mean: ±4.4)

* Hypercholesteremia was induced by intravenous injection of hypercholesteremic rat plasma.

† These increments represent the plasma cholesterol (mg./100 cc.) in *excess* of the pre-injection values.

‡ Number of rats too small to permit calculation of the standard error.

apparently is removed from the blood by the liver, which either destroys it or converts it into cholic acid (30) and perhaps other substances. The rate of disappearance of the excess cholesterol from the plasma of these rats is shown in Table 6 (4). As can be noted, the excess cholesterol disappeared from the plasma more rapidly in the hyperthyroid state, and more slowly in the hypothyroid rats. These results suggested that the hyperthyroid rat was able to destroy or to excrete cholesterol more rapidly than the normal animal, with the converse being true in the hypothyroid rat.

B. *Rate of intestinal excretion of cholesterol*

The feces of 9 hyperthyroid, 10 hypothyroid, and 10 control rats were collected for a 72-hour interval, during which a sterol-free and cholesterol-free diet was given. Analysis then was made of the concentration of cholesterol and of noncholesterol sterols, so that an estimate might be obtained of the actual amount of cholesterol originally excreted as such into the intestine. Analytic methods have been described previously (21). The results are presented in Table 7 (21).

TABLE 7. INTESTINAL EXCRETION OF CHOLESTEROL AND STEROL-DIGITONIDES ON STEROL-FREE DIET

Type of rat	No. of rats	72 hour intestinal collection						
		Dry weight (gms.)	Cholesterol		Non-cholesterol sterol		Total sterol	
			Mgm. %	Mgm./ 72 hrs.	Mgm. %	Mgm./ 72 hrs.	Mgm. %	Mgm./ 72 hrs.
Hyperthyroid	9	4.93 (3.5–6.3)*	454 (370–620)	22 (15–27) ±1.55†	453 (290–770)	22 (13–29) ±1.63	890 (690–1390)	44 (31–56) ±2.52
Hypothyroid	10	1.8 (0.7–2.8)	687 (540–950)	12 (6–20) ±1.28	310 (110–560)	5 (1–9) ±0.9	997 (690–1260)	18 (8–27) ±1.56
Control	10	3.64 (2.2–5.3)	398 (340–500)	15 (9–20) ±1.25	425 (220–840)	15 (8–28) ±1.75	826 (600–1280)	30 (18–38) ±2.57

* Range of values.

† Standard Error of the Mean.

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The hyperthyroid rat was found to excrete a considerably greater amount of cholesterol and noncholesterol sterols than did the normal rat. Since the noncholesterol sterol fraction contains the fecal coprosterols, believed to be derived from cholesterol (31), it can be seen that the hyperthyroid rat excretes more cholesterol than does the normal rat—cholesterol which must be derived from endogenous sources, since the animals received no

dietary sterols during the feces collection period. The hypothyroid rats excreted a considerably smaller amount of noncholesterol sterols than did the normal rats, although the excretion of actual cholesterol was not markedly changed. It is of interest that the actual amount of cholesterol and sterol per gram of stool was not reduced, but that the amount of stool itself was considerably reduced.

C. *Rate of destruction of cholesterol*

The stool assays, although demonstrating a moderate increase in cholesterol and sterol excretion in the hyperthyroid rat, nevertheless did not appear to give results which were pronounced enough to account for the low plasma cholesterol content existing in the face of the known tremendously increased rate of manufacture of cholesterol. Increased destruction of the substance must occur in the body—a process already shown to be possible by earlier investigators (32).

TABLE 8. HALF-LIFE OF CHOLESTEROL IN HYPO- AND HYPERTHYROID RATS

Type of rat	No. of rats	Time $\frac{1}{2}$ (days)
Normal	5	17.3* (14–34.6)†
Hypothyroid	5	43.0 (38.5–69)
Hyperthyroid	5	7.3 (2.9–10.7)

* Median.
† Range.

In order to estimate the rate of this internal destruction, "tracer" studies were performed (7) by injecting water "labeled" with tritium into 5 hyperthyroid, 5 hypothyroid, and 5 control animals, as described previously. Tritium is incorporated from body water into the cholesterol molecule and the rate of loss of labeled cholesterol is then observed. The half-life time ($t\frac{1}{2}$) of the labeled cholesterol can thus be determined (8). In Table 8, it can be noted that the $t\frac{1}{2}$ values were considerably decreased in hyperthyroid rats, and relatively even more markedly increased in hypothyroid rats; in other words, the turnover of cholesterol is significantly accelerated in hyperthyroid rats, with the converse being true in hypothyroid rats. Furthermore, since the plasma cholesterol content of the hyperthyroid rat is decreased, despite a markedly increased rate of cholesterol synthesis, it is clear that the hyperthyroid state is associated with a significantly increased rate of destruction of cholesterol. The converse finding in hypothyroid rats indicates that a markedly depressed rate of destruction of cholesterol is present.

VIII. RELATIONSHIP OF BILE ACID METABOLISM TO CHOLESTEROL METABOLISM IN RATS WITH DERANGED THYROID ACTIVITY

The fact that cholic acid is synthesized *in vivo* from cholesterol, as shown by Bloch and associates (33), and recently confirmed in this laboratory (30), suggested that the metabolism of cholate might affect that of cholesterol. Other studies from this laboratory have confirmed this hypothesis (34–37).

Thus, it was shown that the hypercholesteremia developing after biliary obstruction (34, 35), or following the injection of excess cholate (36) was set in motion by, and dependent upon, a preceding rise in the concentration of plasma bile acids—"hypercholatemia" A similar rise in the level of plasma cholate was found in rats with hypercholesteremia due to experimental nephrosis (38). Moreover, "hypercholatemia" has been found con-

TABLE 9. THE EFFECT OF HYPER- AND HYPOTHYROIDISM UPON THE PLASMA CHOLATE CONCENTRATION

Type of rat	No. of rats	Average weight (Gm.)	Average plasma cholate concentration
			(mg./100 cc.)
Control	13	270	5.6 (3.9– 7.4) S.E.$_m$ ±0.3
Hypothyroid	15	241	7.6 (6.0–11.5) S.E.$_m$ ±0.36
Hyperthyroid	6	195	4.9 (3.0– 6.0) S.E.$_m$ ±0.4

sistently in a variety of clinical states associated with hyper-cholesteremia, including patients with myxedema (37). These findings suggest that the occurrence of hypercholesteremia in some experimental and clinical conditions is a phenomenon *sequential to and dependent*, at least in part, upon a preceding disturbance in cholate metabolism. It seemed important to us to determine whether there was a similar cholate-cholesterol relationship in experimental hyperthyroid and hypothyroid states (18).

Rats were bled and the average plasma cholate concentration was determined. The average plasma cholate was 5.6 mg. per 100 cc. in 13 control animals, 4.9 mg. per 100 cc. in 6 hyperthyroid rats, and 7.6 mg. per 100 cc. in 15 hypothyroid rats. In Table 9 it can be seen that the increase in plasma cholate concentration in the hypothyroid rats, although slight, is statistically significant.

The effect of cholate administration on hypothyroid rats was also stud-

ied. Five of 10 hypothyroid rats were allowed to drink a 0.25 per cent solution of sodium cholate for four weeks. The remaining hypothyroid rats drank ordinary tap water. The average plasma cholesterol level of the 5 rats drinking the cholate solution was 128 mg. per 100 cc. (range: 116–150 mg.; S.E.$_m$ ± 2.9) and that of the controls was 86 mg. per 100 cc. (range: 78–99 mg.; S.E.$_m$ ± 3.4). These results suggest that cholate administration exaggerates the degree of hypercholesteremia found in hypothyroid rats.

IX. MECHANISM OF THE EFFECTS OF THYROID DYSFUNCTION UPON THE METABOLISM OF CHOLESTEROL

The changes just described in cholesterol metabolism probably are not related directly to the changes in metabolic rate. Thus, the plasma cholesterol content is only poorly related to the level of the basal metabolic rate in patients with hyperthyroidism or hypothyroidism, and changes in cholesterol concentration following therapy occur much more rapidly than do changes in the basal metabolic rate (9, 10). Furthermore, clinical diseases characterized by marked changes in metabolic rate are not associated with altered cholesterol levels, nor do the plasma concentrations change when the metabolic rate is increased by the administration of dinitrophenol (39). Moreover, there is no protective action (40) of dinitrophenol, similar to that of thyroid substance, on the plasma cholesterol concentration in animals fed high cholesterol diets. Finally, thyroid administration in nephrotic subjects fails to affect the elevated plasma cholesterol concentrations even when it causes a significant rise of the metabolic rate (41).

It is possible, however, that the cholesterol changes effected by thyroid hormone are due to the direct effects of this substance upon those processes within the cell concerned with cholesterol metabolism. Thus it has been shown that although thyroxine has little effect on cellular permeability, it does appear to accelerate those various enzyme activities *within* the cell concerned with anabolic and catabolic processes (42–45).

X. SUMMARY

The results of these investigations indicate that the hyperthyroid state is associated with a markedly increased rate of hepatic synthesis, destruction, and intestinal excretion of cholesterol. The decreased concentration of plasma cholesterol which occurs in the hyperthyroid state probably is due to the differences in degree of the alterations of synthesis and elimination of cholesterol. Conversely, the markedly depressed rate of hepatic synthesis of cholesterol found in the hypothyroid state probably is associated with hypercholesteremia because of the more marked decrease in rates of destruction and intestinal excretion of cholesterol also present in

this derangement. Thus, the "disparity" between the altered processes of manufacture and of elimination has been shown to underlie the inverse thyroid activity/blood cholesterol relationship. In particular, it appears that the major factor underlying the rise or fall of the blood cholesterol level in hypothyroid and in hyperthyroid states is the altered rate of turnover of cholesterol. In this regard, evidence has been presented which suggests that an alteration of bile acid (cholate) metabolism may play a role.

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ENZYMATIC MECHANISMS OF THYROXINE*

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THE general metabolic effects following the removal of the thyroid gland and replacement therapy by thyroxine are well known and firmly established by many observations on patients and experiments in animals. However, the actual site of action of thyroxine in metabolic exchange in cells is obscure. Barker (1) in his recent review on thyroid mechanisms states that no *in vitro* response to thyroxine has been obtained which gives consistent enough results to be used as a means of testing further the mechanisms of the action of thyroxine. It was in this unsatisfactory state of knowledge that this present work was started. Several actions of thyroxine on oxidative systems have been described (2, 3) and a hypothesis has been formulated for thyroxine action for future work.

The first positive experiment on an enzyme system with thyroxine was derived from a study (2) of the action of this hormone on ascorbic acid oxidase, a copper enzyme from a plant source. Thyroxine stimulated the uptake of oxygen in the ascorbic acid-ascorbic acid oxidase system. This effect was obtained by concentrations of thyroxine as low as 0.9×10^{-6} M. The explanation for this effect was given in terms of a free radical (semiquinone) formation of thyroxine (3). It was postulated that during the course of the oxidation of ascorbic acid, the semiquinone of thyroxine was formed, which could oxidize a molecule of ascorbic acid with the reformation of thyroxine. Huszák (4) postulated a similar mechanism for the stimulatory action of flavonoids on this system. This idea suggests that thyroxine forms a reversible oxidation-reduction system through the action of an oxidase, and, therefore, aids oxidation in this manner. The suggestion that thyroxine can form such a system is not new. For example, Niemann and his associates (5, 6) tested several isomers of thyroxine and found that only the ones which could form a quinoid form had the ability to raise the metabolism of rats. The others, incapable of this mechanism on account of the position of the hydroxyl group, were not active. There-

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fore, they concluded from these experiments that thyroxine worked through an oxidation-reduction mechanism.

Following the work on the ascorbic acid oxidase system, a study was made of the action of thyroxine on the cupric ion catalyzed oxidation of ascorbic acid. This reaction was inhibited by thyroxine (2, 3). Again, micromolar amounts of thyroxine were found to be active in this reaction. The assumption was made that at 50 per cent inhibition one half of the copper catalyst was lost to the reaction by combining with the thyroxine. Calculation showed that 3 molecules of thyroxine were combining with 1 of copper when the velocity of oxidation was reduced 50 per cent.

Following these studies, attention was turned to the oxidations carried out by the cytochrome oxidase system derived from rat's heart. This system in animal tissues has been compared to the ascorbic acid oxidase system in plant tissues and suggestions have been made that the cyto-

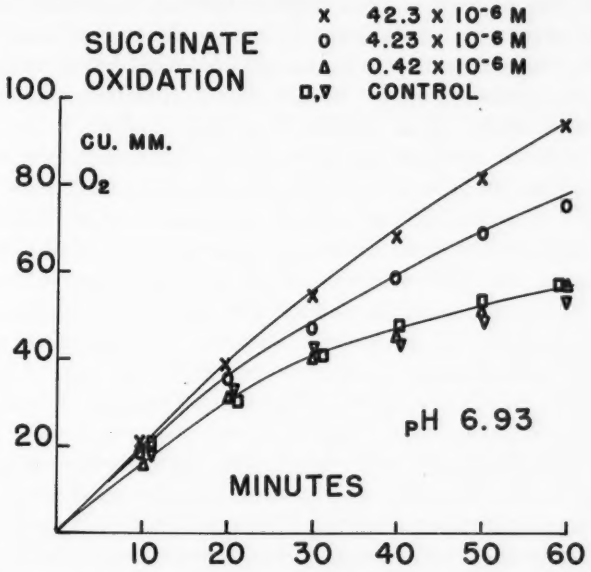

FIG. 1. *The effect of thyroxine on the enzymatic oxidation of succinate.* Abscissae, minutes; ordinates, cu. mm. of oxygen. Concentration of reaction mixtures: 0.4 ml. of water; 1.6 ml. of 0.1 M phosphate, pH 6.87; 0.2 ml. of 1.0 per cent cytochrome *c* in 0.5 per cent NaCl; 0.2 ml. of enzyme solution prepared by grinding 0.252 Gm. of rat heart in 5 ml. of 0.1 M phosphate, pH 6.87, centrifuging and using the supernatant solution; 0.2 ml. of 5 per cent sodium succinate 6 H_2O (in side arm). Thyroxine (Na salt, Bios) prepared by dissolving 25.4 mg. in 1.0 ml. of 0.1 N NaOH and diluting to 100 ml. with water. Final concentrations of thyroxine given in figure. Equivalent amounts of 0.001 N NaOH added to control vessels in place of thyroxine. Temperature of bath, 30.4° C. Final pH 6.93. Total volume in each vessel 3.0 ml.

chrome oxidase system has a copper component (7–10). This suggestion, however, has not been accepted generally (11–13). Also, since thyroxine affects many types of metabolic activity, it is reasonable to assume that the main effect might be on a final common pathway for biologic oxidations.

The chief finding that has come out of this work at the present time is that thyroxine under certain conditions aids in the enzymatic oxidation of succinate by the succinate dehydrogenase-cytochrome *c*-cytochrome oxidase system (Fig. 1). The condition under which this finding was established was through the use of fresh, centrifuged homogenates of rat's heart as an enzyme source. The method of preparation of the enzyme system is to kill the rat by a blow on the head, quickly dissect out the heart, wash in water, weigh, grind in 5.0 ml. of phosphate buffer, centrifuge in an angle centrifuge for five minutes and use the supernatant fluid as a source of the succinate dehydrogenase and cytochrome oxidase. With this preparation, the activity of the oxidative process is reduced and the rate of oxidation of succinate diminishes with time. When thyroxine is present, however, the oxidative activity is not only increased but is prolonged over a much longer period than in the absence of thyroxine. Concentrations ranging from 4.2×10^{-6}M to 42×10^{-6}M gave this effect.

When this work was started, it was thought from visual observation that a concentration of 42×10^{-6}M of thyroxine was fully soluble in these solutions. However, in making up 30-ml. quantities of the solution without enzyme, a faint white precipitate was formed which could be collected by centrifuging the solution. Therefore, it was necessary to control these reactions by using a higher pH, at which the thyroxine remains completely in solution, or to use the centrifuged solutions from which the precipitate was removed before adding the enzyme preparation or the substrate. Both methods gave a positive result with thyroxine on succinate oxidation. The increased activity, therefore, is not due to the presence of a precipitate in the reaction. The increments of oxidation in a given experiment were very constant and depended on the concentration of thyroxine (Fig. 1) although some variation was noted from experiment to experiment.

There have been several references in the literature to the possible relationship of thyroxine to succinate oxidation. Tipton and Nixon (14) have shown an increase in succinoxidase and cytochrome oxidase activity in liver homogenates in rats given thyroxine. This work has been confirmed by Smith and Williams-Ashman (15). Aloisi and Cavallini (16) studied the effects of thyroxine on the succinate dehydrogenase-cytochrome *c*-cytochrome oxidase system and noticed that thyroxine augmented the oxidation of this system when aged or partially purified enzyme systems were used. No effect was noticed with fresh homogenates. Their results were criticized by Smith and Williams-Ashman (15), who stated that in concentra-

tions of 10^{-4}M, thyroxine precipitated out in the flasks. They therefore suggested that the results of Aloisi and Cavallini, which were obtained with much higher concentrations of thyroxine, were due to the presence of the precipitate and were nonspecific. In the present experiments, as has been noted previously, careful attention was paid to this fact and positive results were obtained on the succinate system in concentrations and pH at which the thyroxine remains in solution.

In view of the mechanism proposed to explain the activity of thyroxine on ascorbic acid oxidase (3), it would be of interest to postulate a similar mechanism for the succinate dehydrogenase-cytochrome *c*-cytochrome oxidase system, namely, that through a reversible oxidation-reduction system, thyroxine is acting as a carrier for the succinate system. It has been shown in the present work that methylene blue, a well known carrier, aids in the oxidation of succinate with the centrifuged homogenates but is not as efficient as thyroxine either as to amounts or to prolongation of oxidation. This is circumstantial evidence that thyroxine may be an efficient carrier in this sytem. Potter (17) has defined a carrier as any compound which, by virtue of its ability to be oxidized and reduced, functions in the transport of hydrogen or electrons from tissue metabolites to oxygen. He has set up five criteria for an electron carrier: 1) a natural constituent of animal tissues, 2) capable of reduction at a rate comparable with the rate of oxidation of the substrates, the oxidation of which it is presumed to catalyze, 3) the reduced compound must be capable of being oxidized by tissue preparations at an adequate rate, 4) be catalytically stimulating the rate of hydrogen transport in the system under investigation, and 5) be reduced by one system and oxidized by a second system not identical with the first. Thyroxine is a natural and needed constituent of animal tissues and has been shown in the present work to aid in the oxidation of succinate, which meets requirements 1) and 4). Work is now in progress to examine experimentally the other requirements as given by Potter.

If it can be shown that thyroxine is acting as an electron carrier for the succinate system for all of these requirements, thyroxine would be established in an important place in the main line of biologic oxidations. It would be a mediator between substrates or coenzymes and cytochrome *c* and would aid not only in oxidations but in phosphorylations. Hunter (18) has recently reviewed the relationship of oxidation of succinate to phosphorylation. Martius and Hess (19) in a letter to the Editor of the *Archives of Biochemistry* have suggested that thyroxine may uncouple oxidative phosphorylations. They stated that such an uncoupling may be demonstrated in rats injected with thyroxine. However, they gave as much as 12 mg. of thyroxine to a rat weighing 100 Gm., which is equivalent to

giving 8.4 Gm. to a 70-kilo man. Even with these massive dosages they noted a seasonal variation. In their *in vitro* work the enzyme preparation had to be kept at 0° before use. Variable results were obtained. At the same concentration of thyroxine, both phosphate appearance and disappearance were noted. It is quite obvious that their results do not prove that thyroxine uncouples oxidative phosphorylation.

The question is always raised whether the amount of thyroxine used in *in vitro* experiments is biologic. It is curious that no one ever questions the concentration of enzyme, cofactors, or substrate used in an enzymatic reaction, but if a hormone is added, then the experiment is "biologic" or "nonbiologic" depending on whether or not the concentration of the hormone approximates the normal value needed by man or animals. Barker (1) has summarized the values given for thyroxine in the blood of man and points out that it is between 6 to 10 micrograms per cent and the daily need of a rat is 12 micrograms per kilo. Direct translation of these amounts can not be made to enzymatic systems however. It is therefore necessary to obtain reproducible effects of thyroxine on isolated biologic systems and to use these effects for future thinking and work on this hormone, without defining the reaction as "biologic" or "nonbiologic" based on the blood levels for the hormone in man or the need in animals. However, the dosage needed by man or animals should be used as a guide for obtaining hormone effects in the intact animal.

SUMMARY

Experiments with thyroxine on isolated systems in this laboratory have demonstrated that thyroxine increases the rate of oxidation of the ascorbic acid-ascorbic acid oxidase system (plant origin) and inhibits the cupric ion catalyzed oxidation of ascorbic acid. Thyroxine stimulates the oxidation of succinate in the dehydrogenase-cytochrome *c*-cytochrome oxidase system, using an enzyme preparation from rat heart. Certain postulations have been made from these experiments as to a possible reversible oxidation-reduction mechanism of thyroxine as a guide to future work and thinking.

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THE PHYSIOLOGIC ACTIVITY OF SOME ANALOGUES OF THYROXINE*

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FOR some time we have been assaying the various components of thyroglobulin and some derivatives of thyroxine. Recently we reported on the optical isomers of thyroxine (1) and on the bromine and chlorine analogues (2). The present report is a continuation of this type of study, which has a twofold purpose: 1. To learn of the rôle played by the several structural components of thyroxine in determining its physiologic activity. 2. To search for a compound which might depress the pituitary and the thyroid without increasing the oxygen consumption too greatly.

Of the compounds assayed and reported subsequently in this article, the side chain is altered in two (N-acetyl *l*-thyroxine and N-acetyl *dl*-thyroxyl *l*-glutamic acid), the hydroxy group is substituted in one (methyl ether of thyroxine), the oxygen coupling of the two benzene rings is substituted by sulphur in one (sulphur analogue of thyroxine), and in one the position of the iodine atoms is altered (2′6′-diiodothyronine).

METHOD AND MATERIAL

Each compound was assayed in patients with spontaneous myxedema, N-acetyl *l*-thyroxine in 3 patients, N-acetyl *dl*-thyroxyl *l*-glutamic acid in 2, the methyl ether of thyroxine in 2, the sulphur analogue in 3 and the 2′6′-diiodothyronine in 1. The material was administered daily intravenously[1] and the resulting metabolic changes were compared with those observed in our standard assays of thyroxine polypeptide. In assaying each compound an attempt was made to adjust the dosage so as to approximate the metabolic response to our standard assay of thyroxine polypeptide, given in a dosage of 0.5 mg. of iodine.

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[1] The methyl ether of thyroxine was administered orally as the sodium salt.

RESULTS

The results of the assay of N-acetyl *l*-thyroxine are shown in Figure 1. Analysis of the data indicates that 2.5 to 3.0 mg. of N-acetyl *l*-thyroxine is equal in potency to 1.0 mg. of thyroxine polypeptide (0.5 mg. iodine) or 0.4 mg. of *l*-thyroxine (0.25 mg. iodine). In other words *l*-thyroxine is five or six times as active as N-acetyl *l*-thyroxine.

In Figure 2 are shown the results of two assays with N-acetyl *dl*-thyroxyl *l*-glutamic acid. One patient (J.O'M) made a standard response from a basal metabolic level of −53 per cent to a level of −7 per cent in thirteen days, with a dosage starting with 4.0 mg. and increased to 8.0 mg. on the sixth day. The other patient (A.B.) made a substandard response with a dosage almost twice as great. Although one cannot be exact about the relative potency of this compound, it can be seen that between 6.0 and 10.0 mg. of N-acetyl *dl*-thyroxyl *l*-glutamic acid is equivalent to 1.0 mg. of thyroxine polypeptide. Taking into account the higher molecular weight of the former, it may be said that it has 12 to 14 per cent of the

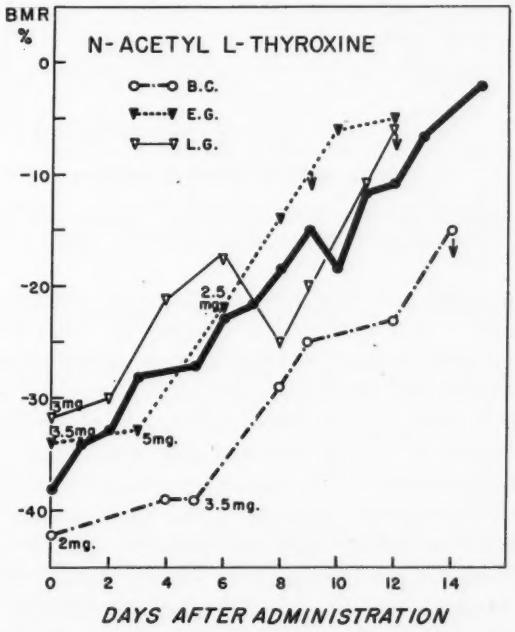

FIG. 1. The calorigenic response of 3 patients with myxedema to various dosages of N-acetyl *l*-thyroxine. The solid black line represents our standard curve of response to the administration of thyroxine polypeptide in a dosage of 0.5 mg. of iodine daily. Small arrows indicate day of final dose.

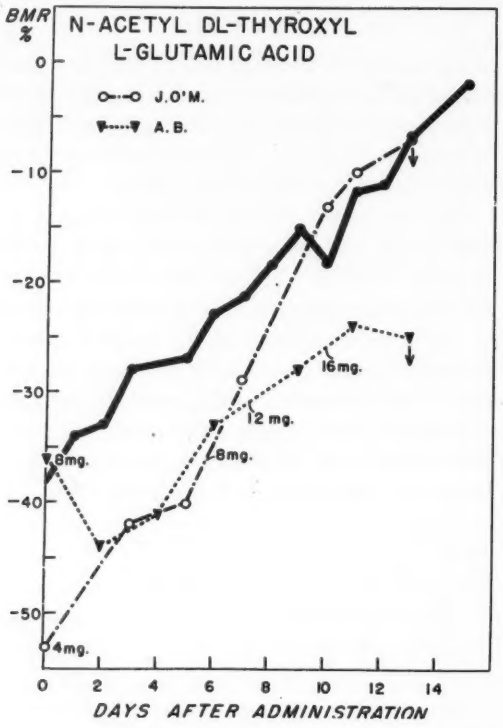

FIG. 2. The response of 2 patients with myxedema to various dosages of N-acetyl *dl*-thyroxyl *l*-glutamic acid. The standard curve is the same as in Figure 1.

activity of thyroxine polypeptide, or 5 to 6 per cent of the activity of *l*-thyroxine.

The results of assay of the methyl ether of thyroxine are shown in Figure 3. The first patient (M. Mc) received a dose of 0.5 mg. initially, which was increased to 1.0 mg. on the eleventh day and to 2.0 mg. on the eighteenth day. The response was substandard. The other patient (I. S.) received a dose of 1.0 mg. at the outset, increased to 2.0 mg. on the fifth day and to 4.0 mg. on the ninth day. The response was slightly better than that of the first patient, but was also substandard. It is estimated, from inspection of the graph, that about 8.0 mg. daily of the methyl ether of thyroxine orally would produce a standard response. Consequently, the methyl ether of thyroxine is about 12 per cent as active as thyroxine polypeptide, or 5 to 6 per cent as active as *l*-thyroxine.

Results of assay of the sulphur analogue of thyroxine are shown in

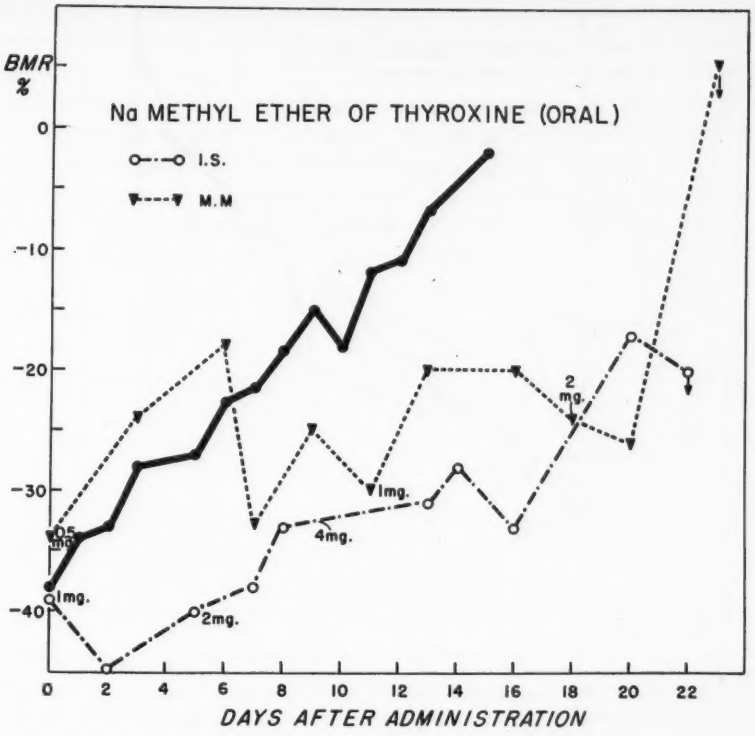

FIG. 3. The calorigenic response of 2 patients with myxedema to various dosages of sodium methyl ether of thyroxine. The standard curve is the same as in Figure 1.

Figure 4. The average daily dose of about 3.0 mg. produced a standard response. Consequently, the activity of this compound is about 30 per cent that of thyroxine polypeptide or 12 to 14 per cent that of *l*-thyroxine.

The compound, 2′6′-diiodothyronine was assayed in 1 patient with spontaneous myxedema in a daily dosage of 50.0 mg. intravenously for nine days. The basal metabolic rate and weight did not change at the end of that period and the blood cholesterol level rose from 443 mg. to 504 mg. per cent. Taking into account the lower molecular weight of diiodothyronine, it seems that a dosage of diiodothyronine two hundred times as great as an equivalent amount of *l*-thyroxine has no physiologic activity.

It should be pointed out that in all but the last case, the patients responded clinically. As the metabolism rose, the blood cholesterol level dropped and the serum protein-bound iodine level, when determined, rose to normal values.

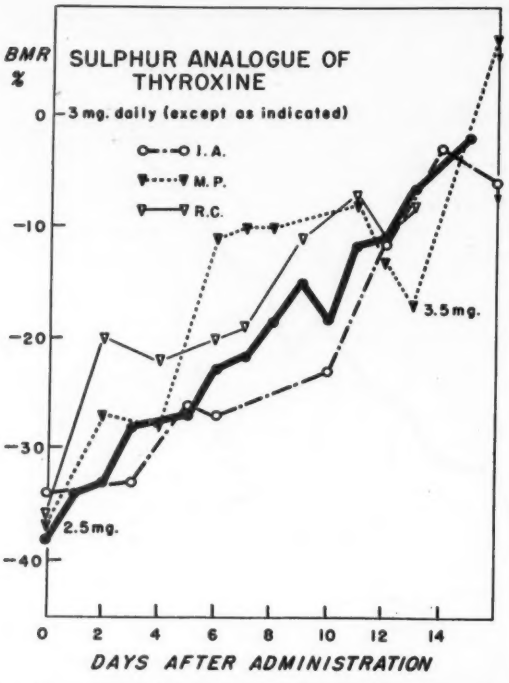

FIG. 4. The calorigenic response of 3 patients with myxedema to various dosages of the sulphur analogue of thyroxine. The standard curve is the same as in Figure 1.

DISCUSSION

As indicated in previous work, the diphenyl skeleton is important in determining the physiologic activity of thyroxine, although thyronine itself, which is thyroxine without the iodine atoms, has little or no activity. Diiodotyrosine has very little physiologic activity, whereas 3,5-diiodothyronine has about 1.5 per cent of the activity of *l*-thyroxine. The foregoing results with 2′6′-diiodothyronine indicate the importance of the position of the iodine atoms (Fig. 5).

Previous work (2) has indicated that substituting bromine or chlorine for iodine diminishes the activity of the thyroxine molecule considerably. Thus, tetrabromthyronine has 3 per cent and tetrachlorthyronine has 0.2 per cent the activity of *l*-thyroxine. According to Lipmann and DuToit (3) the tetranitrothyronine derivative is even less active—about 0.07 per cent as active as *l*-thyroxine.

These results indicate that alterations in the side chains do not alter the activity too greatly. Thus, N-acetyl *l*-thyroxine is about 20 per cent as

active, and N-acetyl *dl*-thyroxyl *l*-glutamic acid is about 6 per cent as active, as *l*-thyroxine. Alteration in the hydroxy position, such as methyl ether of thyroxine, also does not affect the activity too greatly. Thus, the racemic form has about 6 per cent of the activity of *l*-thyroxine. However, according to the review by Niemann (4), replacement of the alanine side chain causes marked reduction in the activity of the molecule.

The replacement of the oxygen in the ether linkage by sulphur does not alter the activity too greatly. Thus, the racemic form of the sulphur analogue has about 10 per cent of the activity of *l*-thyroxine.

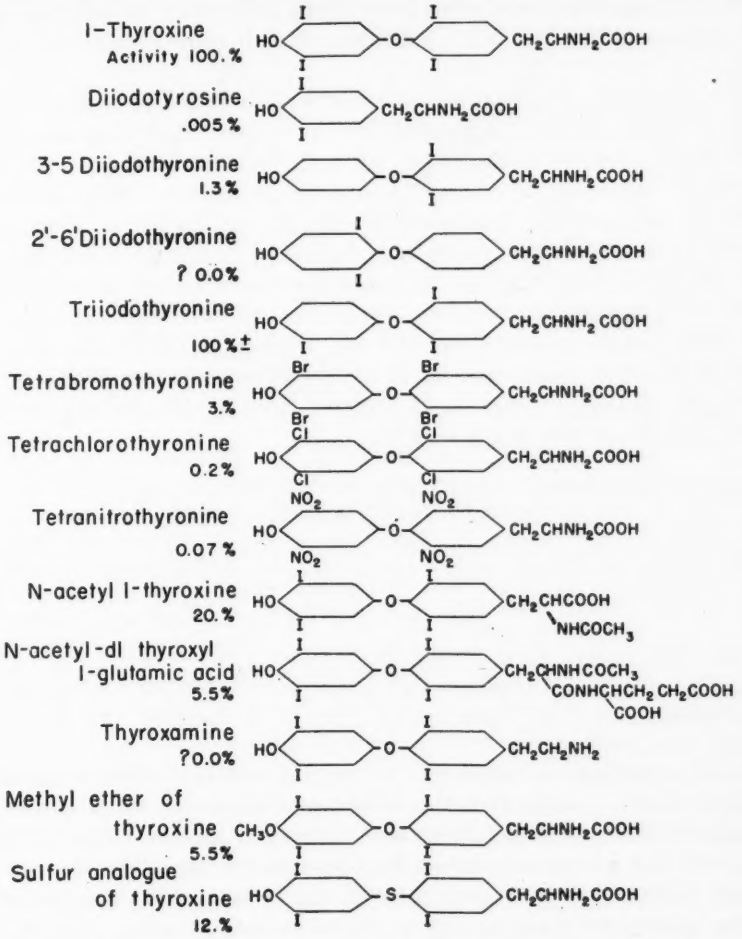

FIG. 5. The structure and relative activity of various isomers and analogues of thyroxine as compared with *l*-thyroxine.

Up to date, our experience has been that any alteration in the structure of the thyroxine molecule results in a compound with diminished biologic activity. It is, therefore, surprising to learn that recently two derivatives have been described with activity greater than *l*-thyroxine. One of these, triiodothyronine, was found by Gross and Pitt-Rivers (5) to be a normal constituent of thyroid tissue and of blood. They demonstrated (6) that by the goiter-prevention test it is three to four times as active as *l*-thyroxine. It is now being tested in patients with myxedema. The other compound, *l*-3′5′-dimethyl 3,5-diiodothyronine was described by Winzler (7) as being 50 per cent more active than *l*-thyroxine in inducing tadpole metamorphosis. It has not yet been tested in other animals or in humans.

Since one of the major purposes of this work is aimed at finding a compound to depress the pituitary and thyroid without increasing the calorigenic effect, we have administered some of these compounds to patients with hyperthyroidism. In a previous paper (2) we reported that tetrabromthyronine administered to a patient with Graves' disease in a dosage of 5 to 20 mg. daily for twenty-nine days, caused an indefinite drop in metabolism but a marked drop in the concentration of serum protein-bound iodine from 24γ to 8.8γ per cent. Since then we have given another patient with Graves' disease 75 to 100 mg. of tetrachlorthyronine daily for a period of eleven days. The basal metabolic rate did not vary but the serum protein-bound iodine level dropped from 39–40γ per cent to 17γ per cent on the twelfth day (Table 1).

TABLE 1. EFFECT OF TETRABROM- AND TETRACHLORTHYRONINE ON PROTEIN-BOUND IODINE IN GRAVES' DISEASE

	Dosage	Duration (days)	Protein-bound iodine	
			Before medication	After medication
Tetrabromthyronine	5–20 mg.	29	24γ%	8.8γ%
Tetrachlorthyronine	75–100 mg.	11	39–40γ%	17.γ%

Since, according to Niemann (4), Cortell's data indicate that 2′6′-diiodothyronine antagonizes the action of thyroxine, we administered 2′6′-diiodothyronine to a patient with Graves' disease in order to determine if it had an anti-thyroxine effect in humans also. The dosage was 4.3 mg. daily, which is equivalent to 2.0 mg. of iodine. The dosage had to be low enough to avoid an iodine remission. After twenty days of this medication there was no evidence of clinical or metabolic change in the patient.

It is worth noting that in the course of our assay, 1 patient with myxedema made little or no response to N-acetyl *l*-thyroxine in a dosage of 3 to 5 mg. for twelve days (Fig. 6). This was unusual, since 3 other patients (Fig. 1) had responded satisfactorily to this dosage. Ten days after the last dose of N-acetyl *l*-thyroxine this patient was given 0.75 mg. of ordinary *dl*-thyroxine intravenously daily for six days, without any metabolic

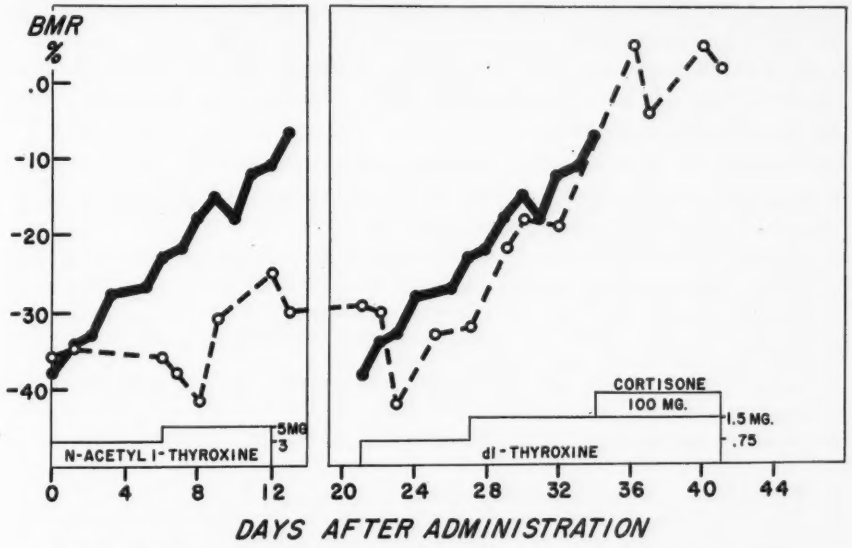

FIG. 6. The left portion illustrates the failure of a patient with myxedema to respond to N-acetyl *l*-thyroxine; the right shows the failure of this patient to respond to *dl*-thyroxine in amounts equivalent to the standard of thyroxine polypeptide (solid black line). The response is partial when *dl*-thyroxine is doubled, but is adequate only when cortisone is administered simultaneously.

change, but after six more days of 1.5 mg. of *dl*-thyroxine there was a moderate rise in metabolism. At this point, cortisone in a dosage of 50 mg. twice a day was added and the same dose of thyroxine was continued for another seven days. During this latter period the metabolism rose speedily to normal values; there was a rapid weight loss, whereas previously the weight was stationary; and the patient improved clinically. This type of response needs confirmation. It may have an important bearing on the fundamental mechanism of the action of the thyroid hormone in the cell. It is somewhat analogous to the situation described by Wolfson *et al.* (8), *viz.*, that with the development of "corticogenic hypothyroidism" the response to a constant dose of ACTH or cortisone is diminished, but there is prompt improvement upon the addition of thyroid.

SUMMARY AND CONCLUSIONS

1. Alterations in the side chain, substitution of sulphur in the ether linkage and substitution of a methyl group in the hydroxy position, do not alter the activity of thyroxine greatly.

2. Substitution of other halogens for iodine does affect the activity considerably.

3. Changing the position of the iodine atoms also interferes with thyroxine activity seriously.

4. The pituitary and the thyroid are depressed by the administration of these relatively inactive thyroxine derivatives but only in proportion to their calorigenic activity.

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THE PATHOLOGIC EFFECTS OF I^{131} ON THE NORMAL THYROID GLAND OF MAN*†

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INTRODUCTION

RADIOACTIVE iodine (I^{131}) is now receiving widespread trial for the therapy of thyrotoxicosis, carcinoma of the thyroid and intractable angina pectoris and congestive heart failure. Although its therapeutic efficacy in many patients with these conditions has been adequately demonstrated, only fragmentary knowledge of the histologic effect of I^{131} radiation on thyroid tissue, normal and pathologic, is available in man. Shapiro (1), Williams *et al.* (2) and Chapman and Evans (3) have reported the radiation effects observed in a small number of thyroid biopsy specimens obtained in thyrotoxic patients treated with I^{131}. Acute cellular injury, noted shortly after therapy, was followed in several months by an increase in fibrous tissue and subsequently by pronounced fibrosis and "regenerative hyperplasia."

In the course of our studies with I^{131} in the induction of hypometabolism in euthyroid patients with intractable angina pectoris and congestive heart failure (4–6) we have been afforded the opportunity to examine the thyroid gland and other tissues in 16 euthyroid paitents who died at varying intervals after I^{131} treatment. These observations of the pathologic effects of I^{131} and the correlation with the delivered radiation, form the basis for this report.

REVIEW OF THE LITERATURE

The pathologic effects of roentgen rays and of radium have been previously reviewed by Warren (7). Although wide clinical experience on the use of roentgen rays and radium in the treatment of diseases of the thyroid has been accumulated, little information is available concerning their histo-

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logic effects on thyroid tissue. Studies of the effect of I^{131} on thyroid tissue have been largely carried out in animals. Hamilton (8) first reported the complete destruction and replacement by fibrous tissue of the thyroid gland in dogs after the administration of 300 microcuries of I^{131} per Kg. Subsequent studies in rabbits (9), mice (10–13) and rats (14–16) have described similar pathologic changes which have varied in character and extent with the radiation dose and interval after administration of I^{131}. With large doses (50,000–300,000 REP) hyperemia, swelling and vacuolization of epithelial cells have been observed within twelve hours after I^{131}. After twenty-four hours, progressive edema, nuclear damage, polymorphonuclear infiltration, follicular disintegration and vascular damage have been noted. There is subsequent complete disorganization of the thyroid gland with eventual replacement in six to eight months by a narrow band of hyalinized fibrous tissue. With smaller doses of radiation, survival of some follicles and epithelial regeneration (in rats but not in mice), particularly at the periphery of the thyroid, has been noted. On the other hand, 12,000 REP in the rat (15) or 1 microcurie per milligram in mice (11), a dosage comparable to that utilized successfully in the treatment of thyrotoxic patients, has failed to produce notable effects on the thyroid gland in these species.

Significant damage to contiguous neck structures, the parathyroids and the trachea (12, 15) has been observed after I^{131} in the mouse and rat. This damage is consequent to irradiation from I^{131} and has been ascribed to the small size of the thyroid gland in these species, relative to the range in tissue of the beta ray of I^{131}. Tumors of the trachea and pituitary gland have also been described in these animal species, occurring from one to two years after I^{131} administration (17). These data emphasize the importance of direct observation in man of the pathologic effects of I^{131}.

CASE HISTORIES

Case 1. D. C., BIH #93141, a 51-year-old man, entered the hospital critically ill with rheumatic heart disease, intractable congestive heart failure and massive anasarca. Nine months later, on March 14, 1947, he received 17 millicuries of I^{131}. Seven per cent was excreted in the urine during the following seventy-two hours; the total urinary output in that period was only 800 cc. No thyroiditis was noted. He expired seven days after administration of I^{131}, with pulmonary edema.

Postmortem examination

Pathologic diagnoses were: healed subacute bacterial endocarditis, mitral stenosis and insufficiency, aortic stenosis and insufficiency, dilatation and hypertrophy of the heart (cor bovinum), thrombosis of the left descending and right coronary arteries, fibrosis of the left ventricle, chronic passive congestion of the viscera, bilateral hydrothorax and bronchopneumonia.

Thyroid and neck structures: The thyroid gland weighed 34 grams and was of normal

size, shape and consistency; there were no nodules or tumors. The I^{131} content was 40 per cent of the administered dose. The mucous membrane of the trachea was injected, and dusky red-grey.

On microscopic examination, the thyroid showed no necrosis, interstitial fibrosis or cellular infiltration. The epithelium was flat and the follicles were filled with pink homogeneous colloid.

Comment: The significant finding in this case was the absence of evidence of any radiation effects in the thyroid seven days after the administration of 17 millicuries of I^{131}, estimated to have delivered 14,500 REP to the thyroid gland during the patient's life. Clinically, also, there was no evidence of thyroiditis.

Case 2. G. S., BIH #M20733, a 42-year-old man, had rheumatic heart disease for twenty-six years and had suffered incapacitating congestive heart failure and repeated bouts of acute pulmonary edema for two years before receiving I^{131}. The 24-hour thyroid uptake following 26 millicuries of I^{131} administered on June 1, 1951, was 31 per cent. The urinary excretion was 57 per cent. No acute thyroiditis was noted during life. The patient expired with acute pulmonary edema on June 8, 1951, seven days after administration of I^{131}.

Postmortem examination.

Pathologic diagnoses were: rheumatic heart disease with calcific aortic stenosis, cardiac hypertrophy, chronic passive congestion of the viscera, and bilateral pulmonary edema.

Thyroid: The thyroid gland weighed 18.5 grams. Direct measurement of the radiation from the dissected thyroid gland showed it to contain 29 per cent of the administered dose of I^{131}. There was no evidence of fibrosis, nodules or tumors.

Multiple sections from five areas were examined. For the most part, the normal histologic structure was maintained. The follicles were well filled with deeply staining eosinophilic colloid and were lined with low cuboidal epithelium. Rare follicles showed detachment of the lining epithelium and fragmentation of colloid. In each section, several areas were noted in which the normal thyroid tissue had been replaced by fibrous tissue with scattered focal collections of small round cells. The fibrous tissue was generally light and fibrillary, though in some areas it was dense and collagenous. There were no remnants of degenerating follicles in the substance of this scar nor any evidence in the parenchyma elsewhere of active necrosis, inflammatory reaction or radiation effect.

Neck structures: The larynx and trachea were normal. Microscopic examination of a section from the trachea underlying the thyroid showed no significant change.

Adrenals: No gross or microscopic abnormalities were noted.

Testes: Both testes and epididymes appeared normal. On histologic examination moderate depression of maturation was noted. No other abnormalities were found.

Pituitary: The pituitary was smaller than usual but normal in appearance.

Comment: In this patient, as in Case 1, the most striking finding was the absence in the thyroid of evidence of radiation effect seven days after the administration of I^{131} estimated to have delivered 31,500 REP to the thyroid gland during the patient's life. The fibrous scar in the thyroid gland antedated the administration of I^{131}.

Case 3. B. R., BIH #M18905, a 65-year-old man, had a subtotal thyroidectomy followed by external radiation for carcinoma of the thyroid, two and a half years before I^{131}. A roentgenogram showed extensive infiltration of the lung fields and a large supraaortic mass, consistent with thyroid metastases. The 24-hour thyroid uptake following

59 millicuries of I^{131} on March 14, 1951, was 32 per cent. Screening of lung fields suggested slight uptake by metastases. The patient entered the hospital critically ill, pursued a downhill course and expired fourteen days after the administration of I^{131}.

Postmortem examination

Pathologic diagnoses were: small-cell carcinoma of thyroid with metastases to lung, mediastinum, pleura, brain; right middle-lobe and left lower-lobe pneumonia; and cystitis.

Thyroid and neck structures: The trachea presented no abnormalities. Residual thyroid tissue on the right, measuring $3 \times 1.5 \times 0.5$ cm., was firm and mottled red-white. A mass of tumor tissue measuring $3 \times 2 \times 1.5$ cm., was noted to the right of the cricoid cartilage, continuous with the mediastinal metastasis below.

On microscopic examination, there was extensive distortion and destruction of the normal thyroid architecture, maximal at the center and progressively less marked as the periphery of the gland was approached. In the areas of maximum destruction the colloid had disappeared. The follicular mass was acellular, represented by an amorphous pink material surrounding an eosinophilic stringy fibrinoid substance and many red blood cells. There was marked infiltration with polymorphonuclear leukocytes and a few small round cells. The stroma was edematous, swollen and showed hyaline degeneration. The walls of the blood vessels, both arteries and veins, showed marked fibrinoid degeneration, intimal hyalinization and antemortem fibrin thrombi; some were ruptured and extravasation of red blood cells was common. At the periphery where the damage was less acute the basic architecture had been maintained. Some follicles still contained colloid which was pale and occasionally fragmented, with numerous droplets which, on Sudan staining, were shown to be fat. However, in most follicles the lining epithelium was vacuolated and swollen; in many areas it had become detached and rested within the follicular lumen. There was increased vascularity and hemorrhage; even in these areas there was hyalinization of the intima of blood vessels and fibrinoid degeneration of the stroma.

Pituitary: The pituitary was normal in size, shape and appearance and weighed 1.9 grams. On microscopic examination there appeared to be a slight increase in the fibrous stroma. There was no evidence of necrosis or tumor. There was some vacuolization of the eosinophilic and chromophobe cells.

Testis: The two testes weighed 40 grams. They were normal in size, shape and consistency. On microscopic examination there was no necrosis, fibrosis or tumor. Spermatogenesis was incomplete; there was mild atrophy.

Comment: Extensive thyroid damage characteristic of irradiation was produced in this case by 59 millicuries of I^{131}, delivering approximately 105,000 REP. Centrally there was almost complete destruction of thyroid tissue accompanied by marked vascular damage. At the periphery, however, the injury, although pronounced, was less marked, and a semblance of thyroid architecture remained. The other endocrine organs showed no changes attributable to I^{131}.

Case 4. A. M., BIH #M17747, a 50-year-old man, had suffered myocardial infarctions eight years, and two and a half years prior to I^{131} therapy. Angina pectoris, present for eight years, had increased markedly in frequency and severity during the two months before I^{131}. Five days after 26.5 millicuries of I^{131} moderate thyroiditis was experienced, which subsided during the next week. Two weeks after I^{131}, increased anginal pain and myocardial infarction occurred. He expired twenty-four days after the administration of I^{131}; approximately 70,000 REP had been delivered to the thyroid gland.

Postmortem examination

Pathologic diagnoses were: acute posterolateral myocardial infarction, coronary arteriosclerosis and marked narrowing of all main stems, myocardial fibrosis, cardiac hypertrophy, pulmonary edema, pleural effusion, bronchopneumonia, and renal infarct.

Thyroid: The thyroid gland weighed 10 grams. There was no gross evidence of fibrosis or tumor.

Multiple sections from four areas were examined histologically. There was extreme distortion of the normal architectural pattern. The destruction was maximal in the center of the gland and less extreme at the periphery. In the center an apparent increase in the fibrous stroma was noted. Most follicles had been destroyed and both colloid and cells were replaced by masses of eosinophilic fibrinoid material and infiltrated with dense collections of polymorphonuclear neutrophils. The areas of most massive destruction of thyroid tissue suggested acute infarction. In other regions hemorrhage had occurred into follicles and into the fibrous stroma. In other areas the polymorphonuclear infiltration was less intense and small round cells were seen. Other follicles, less severely damaged, retained their basic pattern. In these, however, normal colloid was absent and the lumen was occupied by vacuolated colloid and cells which had been disrupted from the follicular margin. Some of these cells were large, round and vesicular; others were pyknotic. At the periphery a few individual follicles and a few groups of follicles with apparently normal morphology were noted; the colloid in these was homogeneous and deeply eosinophilic; the lining consisted of intact, low cuboidal cells. Occasional groups of relatively intact follicles were surrounded by the dense stroma of fibrous tissue. Even within such "nodules," however, there was some evidence of inflammatory cell infiltration, destruction of follicles and vacuolation and swelling of the epithelium, characteristic of radiation injury.

Throughout the gland there was extensive blood vessel damage. The walls of the small vessels were swollen, vacuolated, and showed fibrinoid degeneration and hyalinization. Occasional antemortem thrombi were seen.

Neck structures: The tracheal mucosa was free of injection and appeared grossly normal. On microscopic examination, the tracheal epithelium, subepithelium, glands, connective tissue, and vessels were normal. There were mild degenerative changes of the cartilage.

Comment: Twenty-four days following the delivery of 70,000 REP to the thyroid gland, extensive central necrosis, acute infarction and an acute vasculitis were noted. Peripherally the injury was less acute and some follicles appeared normal. The other endocrine organs showed no change.

Case 5. L. S., BIH M1326, a 52-year-old man had angina pectoris for twenty years. It had been severe for six years, and had culminated in status anginosus during the two weeks before I^{131} therapy. Four doses of 17, 17, 15.5 and 17 millicuries, respectively, were administered on July 23 and 30 and August 10 and 26, 1948. The respective 72-hour urinary excretions were 74, 95, 88 and 86 per cent of the administered dose. No thyroiditis was noted. The patient expired suddenly of an acute myocardial infarction on August 31, 1948, five weeks after the first dose and five days after the last dose of I^{131}.

Postmortem examination

Pathologic diagnoses were: coronary arteriosclerosis, one old occlusion of the left main coronary artery, miliary ischemic necrosis of the left ventricle and of the interventricular septum, possible miliary infarction of the left ventricle, mild myocardial fibrosis, and pulmonary edema.

Thyroid: The normally shaped thyroid was small and slightly firmer than usual; it weighed 12.5 grams. On section it was pale but of normal appearance. There were no nodules or tumor.

Eight sections were examined microscopically. Approximately one fifth of the gland was occupied by normal follicles in which the colloid stained uniformly pink; the epithelium was low and flat. In the remainder, however, the normal follicular pattern had been replaced by loose vascular fibrous tissue in which scattered follicles were seen in various stages of degeneration. In some, colloid was markedly decreased or absent; in others, the epithelial lining had become disorganized and the lumen was occupied by clumps of cells with foamy cytoplasm, apparently derived from the follicular wall, containing a golden pigment. In a few places the follicles were no longer visible; a homogeneous eosinophilic substance which appeared to be colloid, lay free in the fibrous tissue. There was extensive focal small round-cell infiltration, occasionally surrounding the remnants of a thyroid follicle. Foreign-body giant cells were evident. The nerves and blood vessels appeared normal.

Neck structures: The larynx, vocal cords, cartilages and trachea were normal.

Parathyroids: Three small, light brown-gray parathyroids of normal consistency were found. Microscopic examination revealed the usual arrangement of cords and strands and normal oxyphil and clear cells.

Pituitary: The pituitary weighed 0.6 Gm. It was of normal appearance. On microscopic examination there was no necrosis, fibrosis or tumor. The cellular distribution was normal. There was some vacuolization of the basophils. One small focal hemorrhage was noted.

Adrenal: The adrenal glands were normal in size and shape. They showed a normal yellow cortex and a mahogany-brown medulla. On microscopic examination the usual architecture was observed. There was slight fatty infiltration, but no other abnormalities. No necrosis, or fibrosis was noted.

Testes: The testes were of normal size, shape and consistency, although paler than usual. There was no abscess, fibrosis or tumor. On microscopic examination, the testes were normal.

Comment: The striking finding in this case was the presence in the thyroid of acute destruction of follicles, desquamation of follicular epithelium, pigment-containing and foam cells, disruption of colloid, extensive round-cell infiltration and increased fibrous tissue. The thyroid gland, five weeks after the first dose of I^{131}, showed the most extensive round-cell infiltration which was encountered in the series. Other areas of the gland and the blood vessels showed no significant abnormality. The pituitary, parathyroids and other endocrine glands showed no significant change.

Case 6. W. S., BIH #M13290, a 45-year-old man, had a myocardial infarction, followed by severe angina pectoris four years before I^{131} therapy. Propylthiouracil in a dose of 300 mg. daily was administered for one month, followed by two doses of 24.5 and 22.4 millicuries of I^{131}, respectively, on July 22 and 31, 1950; the 24-hour thyroid gland uptakes were 28 and 5 per cent, respectively. A radiation dose of approximately 41,000 REP was delivered to the thyroid gland. Only mild thyroiditis occurred. Increasing severity of angina pectoris, beginning seven weeks after the first dose, was followed by acute myocardial infarction and the patient expired on September 18, 1952, sixty-two days after the first dose of I^{131}.

Postmortem examination[1]

Pathologic diagnoses were: severe atherosclerosis of the coronary arteries, fresh coronary thrombosis, old healed myocardial infarction, pulmonary edema and hydrothorax, and adenocarcinoma of the prostate.

Thyroid: The thyroid gland weighed 20 grams. Both lobes were hard and cut with difficulty. The surface was faintly granular.

In most areas follicles were lined with flat cuboidal epithelium and were filled with homogeneous eosinophilic colloid. Diffusely scattered areas showed a notable increase in interfollicular connective tissue. In some areas there had been follicular disruption, the lumen containing a debris of colloid and large cells, with oval clear vesicular nuclei. There was no infiltration with polymorphonuclear neutrophils and only sparse collections of small round cells. There was no evidence of vascular damage.

Adrenals and testes: The adrenals and testes were normal.

Comment: Sixty-two days after 41,000 REP administered in the form of I^{131} there was little evidence of radiation damage. Increased fibrosis was present but the residual thyroid tissue showed no evidence of progressive follicular degeneration.

Case 7. J. S., BIH #M1834, a 55-year-old man, had had hypertension for eight years and diabetes mellitus and angina pectoris for four years before I^{131} therapy. An acute myocardial infarction three months before I^{131} was followed by status anginosus. Following the administration of 43 millicuries of I^{131} on June 6, 1948, 48 per cent of the dose was excreted into the urine in the next forty-eight hours; urinary collection, however, had been incomplete. Clinical evidence of thyroiditis was absent. The patient expired following another acute myocardial infarction on August 22, 1948, eleven weeks after the administration of I^{131}.

Postmortem examination

Cardiac pathologic diagnoses were: 1) coronary arteriosclerosis with marked narrowing and old occlusion, 2) old infarct of the left ventricle, 3) marked diffuse fibrosis of the myocardium, and 4) bilateral hydrothorax.

Thyroid: The thyroid was hard, atrophic and weighed 7.5 grams.

Multiple sections from two areas were examined microscopically. There were extensive areas of acute necrosis. In some areas only an amorphous pink mass remained, at the periphery of which the shadows of former follicles and thrombosed arteries and veins could be delineated. Many polymorphonuclear leukocytes and extensive hemorrhage were present in the latter regions. At the periphery of the acute infarction and necrosis, the follicles were in various stages of disorganization and destruction. The lining epithelium had been largely desquamated and clumps of cells and fragments of colloid occupied the follicular lumen. Many large clear cells in the lumen contained a golden yellow pigment. In some areas free colloid or irregular groups of epithelial cells were seen in the increased fibrous tissue stroma. Multinucleated giant cells were present. In scattered areas a few relatively normal follicles lined by low epithelial cells contained eosinophilic normal-appearing colloid. Acute changes were noted in the walls of small arteries with foam cells heaped under the intima. There was hyalinization of the intima of some of the larger vessels; occasionally this was marked and associated with an obliterative endarteritis. In the media of one arteriole, calcium deposition was noted.

[1] Performed by Dr. L. W. Falkenburg, Pathologist, Roger Williams Hospital, Providence, Rhode Island, to whom we are indebted for the sections.

Parathyroids: Only one parathyroid was demonstrated grossly. On microscopic examination a fragment of parathyroid was found attached to the thyroid. The chief cells appeared normal. The oxyphil cells, however, showed slight swelling and vacuolization. There was no necrosis, inflammation or fibrosis.

Neck structures: The larynx, vocal cords, cartilages and tracheal mucosa appeared normal. On microscopic examination the trachea revealed no abnormalities.

Pituitary: The pituitary weighed 1.1 Gm. and appeared grossly normal. The characteristic sections were well demarcated. In the anterior lobe the distribution of cell types was normal. The cells stained well, were not vacuolated and showed no abnormality. There was no necrosis, fibrosis or tumor.

Adrenals: The adrenal glands appeared normal. On microscopic examination the cortex and medulla were well defined; the former showed the usual three zones and appeared normal. There was moderate hyalinization of the walls of the blood vessels of the capsule.

Testes: Microscopic examination of three slides of the testes failed to show any abnormality. Spermatogenesis was active.

Comment: The striking finding in this case was the extensive acute hemorrhagic infarction and necrosis of the thyroid gland, seventy-seven days after 43 millicuries of I^{131}. This may have been due in part to primary radiation effects. However, the marked vascular changes may have contributed to this process. The remainder of the gland showed changes consistent with the 77-day interval from administration of I^{131} to necropsy. Other organs, including the pituitary, revealed no changes attributable to I^{131}. A fragment of parathyroid gland attached to the markedly altered thyroid gland showed slight swelling and vacuolization of the oxyphil cells.

Case 8. R. Q., BIH #99148, an 84-year-old woman, had had hypertension for seven years, congestive failure for five years and angina pectoris for six months before I^{131} therapy. An episode of prolonged cardiac pain was experienced two weeks before administration of I^{131}. On March 10, 1948, she received 25.5 millicuries of I^{131}, and 34 per cent of this dose was excreted in the urine during the subsequent seventy-two hours. Ten weeks later there was no evidence of myxedema. Accordingly, a second dose of 21 millicuries of I^{131} was administered on May 25. On June 25, 1948, fourteen weeks after the first dose and four weeks after the second dose, the patient complained of dyspnea and chills; half an hour later she expired.

Postmortem examination

Necropsy was performed three days after death. The cardiac pathologic diagnoses were: coronary arteriosclerosis, cardiac hypertrophy predominantly of the left ventricle, and generalized ulcerated atherosclerosis.

Thyroid: The thyroid gland weighed 17 grams, was extremely firm, and was unattached to surrounding structures or underlying muscles. There were multiple 1-cm. to 2-cm. nodules in both the right and left lobes. Some consisted of soft, pink pieces of thyroid parenchyma; others had the pale, translucent, homogeneous appearance of colloid nodules. A considerable amount of firm, yellow-gray, fibrous tissue was noted in the intervening areas.

More than sixty sections from eight areas were examined. Marked variation in the microscopic anatomy was observed. In some areas, the parenchyma was replaced by dense fibrous tissue, within which occasional well filled follicles were seen. For the most part, however, there remained only shadows of normal follicles, consisting of desquamated cells within a lumen which showed no lining epithelium or colloid. Some of these

cells were round and pink, others were irregular and had pyknotic nuclei. Marked round-cell and polymorphonuclear-cell infiltration was noted. In some areas small collections of cuboidal cells of relatively normal appearance were seen, but these were not in follicular arrangement or in association with colloid.

One section revealed a large colloid nodule consisting of widely dilated follicles surrounded by a low epithelium, and containing deeply eosinophilic colloid. In a few areas the lining epithelium showed evidence of degeneration. Surrounding the well filled follicles there were, in a few areas, groups of small follicles with the microscopic appearance of fetal adenomata. Significantly, surrounding the nodule, areas of thyroid tissue showed acute destruction with disorganization of follicles, desquamation of the lining epithelium, cellular infiltration and disappearance of colloid.

Another area also demonstrated a well encapsulated, colloid nodule, the follicles of which were bounded by low epithelium and filled with pink homogeneous colloid. Strikingly, however, at the bottom of this nodule, there was a wedge-shaped area of acute destruction with disorganization and disintegration of the follicular wall, pyknosis of the nuclei, fragmentation of the colloid and desquamation of the epithelium bounding the individual follicles. In the lumens of the follicles, clumps of cells were mingled with a granular debris and with some large, round, clearly outlined "foam" cells. In parts of this wedge-shaped area the follicles could no longer be clearly identified. Frequently the follicular walls had disappeared and the colloid had coalesced to form masses. This was accompanied by acute cellular reaction with polymorphonuclear infiltration. Multinucleated giant cells were also noted. This area in a well circumscribed colloid adenoma demonstrated, strikingly, the same process of acute destruction seen after administration of I^{131} in other cases, and in other sections in this case.

Neck structures: The larynx, vocal cords, cartilages and trachea were lined by a smooth, yellow-gray mucosa which appeared normal.

Parathyroids: One parathyroid was clearly identified and was separated from the thyroid by 2 millimeters of normal-appearing fat. On microscopic examination two parathyroids were noted. One parathyroid was found within the substance of the thyroid gland. Despite the fact that immediately adjacent there were areas of acute destruction of thyroid parenchyma, this parathyroid was intact. The other parathyroid showed mild congestion but a normal cellular pattern. Chief cells predominated but there were large numbers of pale oxyphil cells.

Pituitary: The pituitary weighed 2.3 Gm. and appeared somewhat larger than normal. It was normally situated in the sella turcica and was shelled out without difficulty. A tiny cyst, 1.5 mm. in greatest diameter, containing a pale gelatinous colorless fluid, occupied the posterior lobe away from the junction with the pars intermedia. The anterior lobe presented a scattered, nodular, yellow appearance. On microscopic examination the gland, though poorly preserved, was regarded as normal.

Ovaries: On microscopic examination the ovaries were atrophic. The vessels showed intimal thickening, hyalinization and obliterative endarteritis.

Comment: Striking findings in the thyroid gland in this case were: 1) the extensive scar tissue and fibrosis, which had apparently followed the first dose of I^{131}, fourteen weeks previously, 2) the apparent recent involvement of one adenoma, possibly as the result of the second dose four weeks before death; other areas also showed evidence of acute destruction of thyroid tissue with polymorphonuclear infiltration, 3) the survival of a few individual follicles in the mass of scar tissue, 4) the intact parathyroid within the destroyed thyroid, and 5) the large number of colloid nodules and fetal adenomata which were apparently unaffected by I^{131}.

Case 9. M. C., BIH #92290, a 62-year-old man, had suffered a previous myocardial infarction and cerebrovascular accident. Angina pectoris had been experienced for eleven years and congestive heart failure for six months before I^{131} therapy. Propylthiouracil, 200 mg. daily, was administered from January 25 to February 15, 1947. Eight hours after the last dose of propylthiouracil the patient received 17 millicuries of I^{131}; 82 per cent of the I^{131} was excreted in the urine during the following seventy-two hours. Propylthiouracil, 150 mg. daily, was again administered from May 3 to June 2, 1947. Forty-eight hours later, a dose of 17 millicuries of I^{131} was administered; 62 per cent was excreted in the urine during the following seventy-two hours. The patient expired on July 17, 1948, one hundred and twenty-five days after the first dose and forty-three days after the last dose of I^{131}.

Postmortem examination

Cardiac pathologic diagnoses were: coronary atherosclerosis, healed posterolateral myocardial infarct with ventricular aneurysm, myocardial hypertrophy and bilateral hydrothorax.

Thyroid: The thyroid was small and extremely hard. There was a firm nodule in the left upper pole. On section, the gland showed an increased amount of fibrous tissue. Small colloid cysts were present.

Ten microscopic sections from two areas were examined. Scattered through the increased dense fibrous tissue were colloid-containing follicles; the colloid was basophilic, usually decreased in amount and sometimes full of debris and cells. The epithelium lining of the follicles was flat, often apparently absent, and only rarely cuboidal. There were many clusters of cells in a circular arrangement but without a lumen. In the midst of the fibrous tissue and surrounded by it was a large well-encapsulated and well-demarcated involuted nodule, the follicles of which were full of dense, ribboned, pink-staining colloid and lined by cuboidal or flat epithelium. One side of the nodule presented clusters of large eosinophilic epithelial cells (Hürthle type) in follicular pattern but without colloid and frequently without a lumen. There was no evidence of acute necrosis, infiltration, tumor, or vascular change.

Parathyroids: Three brown-red parathyroids were found, measuring 2.0 by 1.0 mm. On microscopic examination no significant histopathologic changes were observed. There was no necrosis, inflammation or fibrosis.

Adrenals: On microscopic examination, although there was some disorganization and vacuolization of the cell columns, significant necrosis or fibrosis was not noted.

Testes: On microscopic examination there was increased fibrosis throughout the organ but not between the individual tubules. Considerable prominence of the interstitial cells was noted. There was no necrosis, infiltration or tumor.

Comment: The striking finding in this case was the extensive and marked interstitial fibrosis of the thyroid and the destruction of most of the normal tissue surrounding a large nodule, after two doses of 17 millicuries of I^{131}. Despite the larger retention of the second dose forty-three days before death, there was no evidence of acute or subacute change in the thyroid. It seems likely that the amount of residual colloid or functional tissue remaining in the nodule or in the few intact follicles was sufficient to maintain a euthyroid state.

Case 10. H. O., BIH #96253, a 62-year-old man, had had rheumatic heart disease for twenty-six years, hypertension for seven years, and congestive heart failure with angina pectoris for one year before I^{131} therapy. On July 5, 1947, he received 17 millicuries of I^{131} and 0.5 mg. of stable iodide as carrier; 51 per cent of the I^{131} was excreted

in the urine during the following seventy-two hours. During the next few weeks no evidence of hypometabolism was noted. On September 18, a dose of 3.9 millicuries of I^{131} was administered. Four weeks later the basal metabolic rate was −29 per cent. Propylthiouracil was administered from October 11 to December 3, 1947. Three weeks later, the basal metabolic rate had risen from −25 per cent to −2 per cent and the serum cholesterol content had decreased. The patient then received 15 millicuries of I^{131}; 48 per cent was excreted in the urine during the following seventy-two hours. Five weeks later the basal metabolic rate was −15 per cent. On February 7, 1948, the patient expired with pulmonary edema, thirty weeks after the first dose and five weeks after the dose of I^{131}.

Postmortem examination

Pathologic diagnoses were: mitral and aortic stenosis and insufficiency, calcification of the aortic and mitral valve, cardiac dilatation and hypertrophy, diffuse myocardial fibrosis, focal ischemic myocardial necrosis, bronchopneumonia, pulmonary venous congestion and pulmonary edema, old splenic infarcts, and extensive fibrosis of the pituitary.

Thyroid: The thyroid gland weighed 5.7 grams. It was firm and there was no evidence of perithyroiditis or adhesions to adjacent structures. On section, it was firm and diffusely fibrosed; there was no evidence of colloid, nodules or tumors.

Eight sections from two areas were examined microscopically. The most striking finding was marked and extensive interstitial hyaline fibrosis, extending into structures that once were acini, and obliterating the previous architecture. Scattered in this dense fibrous stroma were thyroid follicles. A few were of normal size, containing an eosinophilic homogeneous colloid with either flat or absent epithelium; others were abnormally small. Often the colloid was vacuolated or disintegrating into globules. Many follicles contained a granular debris consisting of colloid and shadows of old cells. Cells ejected from the lining membrane lay free inside the follicular lumen, intermingled with a golden yellow pigment. In a few areas only clusters of pleomorphic, pyknotic cells remained as evidence of previous follicles. The vessels showed thickening and hyalinization of the intima.

Pituitary: Nine sections from two areas were examined. Approximately one half of the gland was replaced by widespread areas of focal and diffuse fibrosis. There was a generalized falling out of alveoli and columns of pituitary cells throughout all parts of the gland. Many more eosinophils than basophils remained. A few remaining basophils were large and contained deeply staining granules. Some of the eosinophils stained bright and glaring red. Many cells appeared to form a lobular and alveolar arrangement, often with a tiny lumen containing a hyaline-staining colloid-like material. The eosinophils were frequently distorted and the cellular outlines poorly demarcated. In some areas only the nuclei were left in an amorphous mass of material. There was no evidence of arteriosclerosis, of thrombosis or of hemosiderosis. The findings were consistent with marked fibrosis and atrophy of the pituitary.

Testes: There was no gross or microscopic abnormality.

Adrenals: There were no gross pathologic findings. Microscopically, the cells of the zona fasciculata were filled with lipid, which distorted the architecture of the columns. There was no necrosis, fibrosis or tumor.

Neck structures: The vocal cords, cartilages and tracheal mucosa appeared normal. The tracheal epithelium, normal in appearance, exhibited intact cilia. The tunica propria was slightly edematous and there was a small amount of lymphocytic infiltration of the stroma. The cartilage was normal in appearance. The findings were those of a normal trachea.

The findings in other organs were compatible with the diagnoses listed above.

Comment: The extensive fibrosis and alteration in the colloid of the thyroid gland were striking findings. It is of interest to note that in this patient only transient hypometabolism developed. Presumably the few remaining thyroid follicles were adequate to restore a euthyroid state. Some follicles, however, showed evidence of a subacute or continuing degeneration; no evidence was found of an acute necrosis or cellular infiltration subsequent to the second dose of I^{131}, five weeks before death. The marked fibrosis and atrophy of the pituitary cannot be definitely attributed to I^{131}; the patient had been in shock for prolonged periods, a condition occasionally associated with pituitary necrosis and fibrosis. The pituitary did not show the changes which have been previously described as following total thyroidectomy.

Case 11. H. M., BIH #M16572, a 52-year-old man, suffered myocardial infarctions seventeen and twelve years before I^{131} therapy. Angina pectoris and congestive heart failure, present for eight years, became incapacitating one year before treatment with I^{131}. Four doses of I^{131}, 31.5, 30, 31 and 32 millicuries, were administered on April 18, May 2, July 7 and September 19, 1950, respectively. The 24-hour thyroid gland uptakes were 41, 9, 11 and 20 per cent, respectively. One month after the last dose of I^{131} (six months after the first dose) clinical and laboratory evidence of myxedema developed. The myxedema was controlled with desiccated thyroid 16 mg. daily. The patient expired on February 23, 1951, three hundred and sixteen days after the first dose of I^{131}.

Postmortem examination

Pathologic diagnoses were: cardiac aneurysm with old organized thrombus, myocardial fibrosis, cardiac hypertrophy, coronary arteriosclerosis, pulmonary edema, and bronchopneumonia.

Thyroid: The thyroid weighed 9.2 grams. It was firm, with some distortion of the lobular pattern; on section there were no nodules or tumor.

Multiple sections from four areas were examined microscopically. There was complete obliteration of normal thyroid architecture. The parenchyma had been replaced by dense bands of fibrous tissue. In some areas the arrangement of the fibrous tissue offered a faint suggestion of the original thyroid lobulations. Infrequent small areas were noted with fibroblasts, increased vascularity and collections of small round cells surrounding isolated pink-staining homogeneous material, probably colloid. The original follicles were represented by holes, giving a Swiss-cheese appearance. Most of these contained neither colloid, nor cellular lining; within the lumens of some of them, irregularly shaped, pyknotic, bizarrely staining cells remained free. Occasional large cells with homogeneous pink cytoplasm and large vesicular nuclei rested free, or formed follicles or columns in the fibrous tissue. Elsewhere, follicles contained only an irregular, eosinophilic-staining, homogeneous debris. Other follicle "shadows" contained only eosinophilic strands. The medium sized arteries showed intimal thickening and hyalinization.

Neck structures: The larynx, vocal cords and cartilages were normal. The mucosa of the trachea was injected and covered by thick mucous material.

Adrenals: The adrenals were normal in size, shape and architecture. On microscopic examination no abnormality was noted.

Testes: Each testis weighed 23 grams and appeared normal grossly. On microscopic examination there was marked fibrosis and hyalinization of many tubules and varying degrees of germinal atrophy. Spermatogenesis was inactive and many tubules were completely hyalinized.

Comment: In this case there was almost complete fibrosis and obliteration of the

normal thyroid three hundred and sixteen days after the first dose of I^{131}. Only shadows of the former follicles remained; normal colloid and epithelium were absent and the thyroid vessels were hyalinized. The clinical myxedema observed in this patient was consistent with these findings. The testicular fibrosis and tubular hyalinization was of considerable interest. These changes cannot be confidently attributed to I^{131}. Our previous studies (6) showed a small I^{131} uptake by the testis; and other cases in the present series showed no significant changes. Moreover, this patient before I^{131} therapy had a eunuchoid appearance, smooth fine skin and sparse facial hair.

Case 12. R. S., BIH #M1052, a 69-year-old man, had suffered angina pectoris for eighteen years. Left paravertebral alcohol injection, twelve years before I^{131} therapy, was followed by intercostal neuritis. Thyroid denervation was performed one year after the alcohol injection. Before I^{131} therapy, hypometabolism was manifested by a basal metabolic rate of −20 per cent and a serum cholesterol level of 226 to 260 mg. per 100 cc. Two courses of treatment with pituitary thyrotropin were followed by 30 millicuries of I^{131} on December 1, 1947; 50 per cent of the I^{131} was excreted in the urine during the following seventy-two hours. Six weeks after I^{131}, symptoms of myxedema were noted and were controlled by 6–12 mg. of thyroid daily. In November 1948, three hundred and forty-nine days after I^{131} therapy, the patient expired following a self-administered overdose of chloral hydrate.

Postmortem examination

Necropsy was performed approximately fifteen hours post mortem. The pathologic diagnoses were: marked coronary arteriosclerosis and fibrosis of the myocardium, and focal necrosis of the liver.

Thyroid: The thyroid gland weighed 12 grams. It was small, quite firm and adherent to the underlying tissues. There was no evidence of tumor or nodules. On section, it did not show the characteristic dark red appearance of normal thyroid; the surface was pale pink with grey fibrous bands traversing the parenchyma.

Nine slides from four areas were examined. In most areas the tissue was scarcely recognized as thyroid. The shadow of a follicular pattern remained but no normal colloid was seen. The acini consisted of rings of fibrous tissue lined with only very few remaining cuboidal cells. The lumens of the acini contained free cells, apparently ejected from the wall. Many were fragmented, clumped, and irregular in size and shape. Some cuboidal cells were in arrangements resembling normal acini. There was an increase in connective tissue and many focal collections of small round cells, as well as a diffuse round-cell infiltration. Large multinucleated giant cells were noted. In scattered areas there were clumps of large, brightly staining, eosinophilic cells with hyperchromatic pleomorphic nuclei. In rare areas scattered colloid-containing follicles were separated by dense homogeneous fibrous tissue. The cells lining such follicles were flat. The colloid was eosinophilic and ribboned and occupied only the center of the follicular lumen. In some areas it contained foamy material and a golden yellow pigment. There was no evidence of vascular change.

Neck structures: The tracheal mucosa appeared normal.

Parathyroids: Attached to the thyroid and separated from its markedly altered parenchyma only by the thickness of a connective tissue capsule was normal-appearing parathyroid tissue. The nuclei were dark and uniform in size; the cytoplasm was pale; there was no evidence of fibrosis or necrosis.

Adrenals: On microscopic examination the usual three zones were made out. There were a few small areas of destruction and lymphocytic infiltration. Otherwise the architecture appeared normal.

Testes: The seminiferous tubules were well outlined. Spermatogenesis was present. There were no areas of necrosis, fibrosis or infiltration.

Comment: This patient was hypometabolic at the time of I^{131} treatment. Six weeks after 20 millicuries of I^{131} frank myxedema developed, as evidenced by further lowering of the basal metabolic rate, a rise in the serum cholesterol level and the appearance of clinical signs and symptoms. One year later, the thyroid gland was hard and fibrosed, with almost complete destruction of functioning thyroid tissue. Only a rare colloid-containing follicle remained. The outline of the former follicular structure was visible in most areas, but without normal epithelium or colloid.

Case 13. N. A., BIH #71514, a 43-year-old woman, had rheumatic fever twenty-five years and again six years before I^{131} therapy. Congestive failure, present for twenty-one years, required multiple hospitalizations during the six years before treatment with I^{131}. Polycythemia vera and multiple cerebral embolic episodes occurred during the three months before I^{131} administration. She received 39 millicuries of I^{131} on November 9. 1948, and 48 per cent was excreted in the urine during the following seventy-two hours. Mild thyroiditis was noted during the second week after I^{131} and continued for approximately two weeks. Ten weeks later, there was clinical and laboratory evidence of myxedema, subsequently controlled with 12 mg. of desiccated thyroid daily. The post-I^{131} course was marked by continued severe congestive failure and emboli to the brain, kidney and arm. The patient expired following a cerebrovascular accident on July 18, 1952, five hundred and eight-five days after the administration of I^{131}.

Postmortem examination[2]

Pathologic diagnoses were: rheumatic heart disease with involvement of mitral, aortic, and tricuspid valves; cardiac hypertrophy and dilatation; coronary arteriosclerosis; pericardial effusion, right pleural effusion and ascites; and infarcts of kidney, spleen and brain.

Thyroid and neck structures: The thyroid gland weighed approximately 9 grams. It was small and firm. The larynx and trachea were not remarkable.

Sections from two areas of the thyroid were examined histologically. The normal thyroid architecture had been almost entirely replaced by dense sheets of fibrous tissue. Scattered throughout the stroma were focal collections of small round cells. These were frequently in association with areas of residual degenerating thyroid tissue. In such regions there were remnants of thyroid acini, consisting of irregularly shaped and sized masses of colloid-like material lying in the stroma and surrounded by a single or a few cells. The nuclei of the cells were occasionally large, hyperchromatic, pyknotic, or irregular; but more often, large, pale, clear, and irregular. Two small encapsulated nodules were noted within dense fibrous tissue. Although these showed, more clearly than the rest of the gland, remnants of normal colloid-containing acinar structure, they, too were undergoing necrosis. Destruction of acini, degeneration of the epithelial wall, and fragmentation of colloid were visible within these nodules. There was no polymorphonuclear infiltration or acute vasculitis. The smaller arteries, however, showed hyalinization of the intima and thickening of the walls. The larger vessels showed some atherosclerosis.

Parathyroids: Three parathyroid glands were dissected and appeared normal. On microscopic examination there was no evidence of destruction, infiltration or tumor. Fat and parenchyma were in the usual proportions. Most cells were of the water-clear type.

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[2] Performed by Dr. George F. Meissner, Pathologist, Jewish Memorial Hospital Boston, to whom we are indebted for the sections.

Pituitary: The pituitary weighed approximately 2 Gm. and bulged the roof of the sella turcica. On microscopic examination, the majority of cells were large and pale with vesicular, round or oval nuclei and a homogeneous or slightly granular, pale cytoplasm. In the center, typical distinct eosinophils were seen. In scattered areas distinct basophilic-staining cells were noted. No differential count was performed. There was no evidence of destruction, infiltration, or tumor.

Adrenals: Grossly and microscopically normal.

Comment: This patient received only one dose of I^{131}, nineteen months before death, and had been persistently hypothyroid for seventeen months before death. There were dense fibrous sheets replacing most of the thyroid. Despite the long interval after administration of I^{131}, continued degeneration of the last remnants of thyroid acini and colloid were evident. Moreover, two small thyroid nodules showed destruction of acini, degeneration of the epithelial wall and fragmentation of colloid. It is improbable that these changes were due to the direct effect of I^{131} irradiation. Progressive vascular damage with resultant anoxia and increasing fibrosis are more probable etiologic factors.

Case 14. L. A., a 69-year-old female, had hypertension for eleven years, severe angina pectoris for ten years and myocardial infarctions eight, five, and one year before treatment with I^{131}. Seven doses of I^{131} were administered (Table 1).

Two weeks after the first dose the patient experienced a mild sore throat which lasted about two weeks. The thyroid gland was somewhat tender. A 1-centimeter nodule, readily palpable at this time at the right lower pole, gradually became smaller and could not be felt two months after the first dose. Dramatic relief of angina pectoris was noted during the first year after treatment. Moderate cardiac pain during the second year was followed by increasing angina pectoris, culminating in an acute myocardial infarction. The patient expired on September 6, 1951, eight hundred and sixty-nine days after the first dose of I^{131}.

Postmortem examination

Necropsy was limited to an examination of the heart and the neck structures. Pathologic diagnoses were: acute myocardial infarctions of the right and left ventricles, myocardial fibrosis, and coronary arteriosclerosis with multiple old and fresh occlusions.

Thyroid: The thyroid gland weighed 2 grams. It consisted of white, firm, fibrous tissue.

Multiple sections from six areas were examined histologically. No readily identifiable thyroid tissue could be found. The tissue was composed of fibrillary, or hyalinized, hypocellular, fibrocollagenous and fibro-hyaline material, which stained pale blue to pale pink. Throughout, there were many oval or elongated cells with an eosinophilic cytoplasm which varied from completely homogeneous to granular; these contained large, rounded nuclei without nucleoli. An occasional nucleus was pyknotic. These cells, although quite atypical, were not anaplastic. They occurred singly and also as nests devoid of recognizable acinar arrangement, polarity or lumen. Two microscopic areas found in a careful survey of all six slides, differed. In one of these two areas, eosinophilic colloid was surrounded by flat cells of an appearance similar to "epithelial" cells. In the other area, the colloid was surrounded by continuous cytoplasm with a single elongated nucleus. It cannot be definitely stated whether this represented intraluminal or intracellular colloid. There were no giant cells in the stroma and the stromal cells did not appear abnormal. Throughout the fibrous tissue there were scattered plasma cells and lymphocytes, rarely in aggregates.

Blood vessels of all sizes showed profound changes consisting of fibrous thickening

TABLE 1

	I^{131} Data								
							Gross		
Case No. and initials	Dose (mc.)	Thyroid gland uptake (% in 24 hrs.)	Biologic half-life (days)	Urinary excretion (% in 72 hrs.)	Interval from dose to necropsy (days)	Est. REP delivered to thyroid	Wt. (Gm.)	Fibrosis	Nodules
1. D.C.	17	40*	est. 8	7	7	14,500	34	0	0
2. G.S.	26	31	8		7	31,500	18.5	0	0
3. B.R.	59	32	est. 5		14	105,000	est. 12.5	—	—
4. A.M.	26.5	65**	5.5	33	24	70,000	10	0	0
5. L.S.	17 17 15.5 17 66.5	—	—	74 95 88 86	37 30 19 3	not estimated	12.5	+	0
6. W.S.	24.5 22 46.5	28 5	7.6 1.0	65 73	62 54	41,000	20	+	0
7. J.S.	43	—	—	47+	80	not estimated	7.5	++	0
8. R.Q.	25.5 21 46.5	—	—	34 71	93 31	not estimated	17	++	++++
9. M.C.	17 18.5 35.5	—	—	82 62	125 43	not estimated		++++	+
10. H.O.	17 2.5 25.5 45	—	—	51 48	186 142 42	not estimated	5.7	++++	0
11. H.M.	31.5 30.8 32.0 32	40 9 11 20	8 0.8 0.8 2.2	38 50 61	316 302 236 162	80,500 2,000 5,500 27,000	9.2	+	0
12. R.S.	30.5	—	—	70	349	not estimated	12	+++	0
13. N.A.	39	—	—	48	585		9	++	0
14. L.A.	17 17.5 24 25.5 22 23.5 27.5	12 22 14	0.9 1.1 1.1	60 93 90 93 68 57 60	869 862 840 820 669 567 347	not estimated	2	++++	0
15. M.G.	27.5 22	40 9	5.4 est. 2	64 96	890 882	38,000 2,500 40,500	5.6	++++	0
16. M.G.	27 30.5	—	—	28 36	1,092 1,079	not estimated	4.7	++++	0

Thyroid pathology											
Microscopic											
Acute changes							Chronic changes				
Necrosis	Infiltration		Infarction	Acute vascular damage	Follicular disruption	Loss of colloid	Fibrosis	Chronic vascular changes	Loss of follicular epithelium	Loss of follicular colloid	Regeneration
0	0		0	0	0	0	0	0	0	0	0
0	0		0	0	0	0	0	0	0	0	0
++++	++++	+	++++	++++	++++	++++	0	0	0	0	0
+++	++	+	+++	+++	+++	+++	+	0	0	0	0
+++	0	++++	0	0	+++	+++	++	0	0	0	0
0	0	+	0	0	+	+	+	0	0	0	0
++++	++++	0	++++	++++	++++	+++	+	0	++	++	0
+	+	++	0	0	++	++	++	0	++	++	0
+	0	+	0	0	+	+	+++	0	+++	+++	0
+	0	+	0	0	++	++	+++	0	+++	+++	0
0	0	+	0	0	+	0	++++	++	++++	++++	0
0	0	0	0	0	0	0	++++	+	++++	++++	0
0	0	++	0	0	++	++++	+++	+	++++	++++	0
0	0	0	0	0	0	0	++++	++++	++++	++++	0
0	0	0	0	0	++	++	++	++	++	++	0
	0	0	0	0	++	++	++++	++	+++	++++	0

* Determined by beta method (6).

** Calculated by extrapolation of 48-hour and 96-hour data. The uptake at forty-eight hours was 61 per cent and at ninety-six hours, 59 per cent.

and hyalinization of the intima and usually of the media as well. The elastica interna was sinuous, disrupted, reduplicated and eosinophilic. There was general narrowing and even occlusion of the vessels by the thickened and fibrosed intima and probably by organized thrombi. There were several instances of microscopic sized, "saccular," arteriolar and arterial aneurysms. The thyroid capsule, identified by its looser texture, contained vessels which showed similar changes, though of a less pronounced degree. One vessel showed calcification of the elastica interna and patchy calcification of the media.

Parathyroids: Four sections, one from each gland, were examined histologically. The cells were arranged in sheets and acinar formation, the predominant cell being the chief cell with some nests of oxyphil cells. There was only a small amount of fat present. In two slides there was colloid enclosed by chief cells. There was no fibrosis. The vessels showed some degree of intimal fibrosis, though it was not striking,

Neck structures: The tracheal mucosa consisted of normal respiratory epithelium. A few lymphocytes were present in an edematous submucosa. The laryngeal cartilages and submucosal glands presented no abnormalities.

Comment: Hypometabolism developed in this patient following seven doses of I^{131}, totalling 157 millicuries. Eight hundred and sixty-nine days after the first dose, the thyroid gland weighed only 2.5 grams and consisted of a dense sheet of connective tissue. There was no evidence of the nodule which had been felt and had been noted to regress during I^{131} therapy. Not a single intact thyroid follicle was observed. Scattered epithelial cells were noted in the dense fibrous stroma. The walls of the vessels showed considerable hyalinization and thickening. No abnormalities were noted in the parathyroid glands.

Case 15. M. G., BIH #M25408, a 71-year-old man, had myocardial infarctions nine and five years before I^{131} therapy and severe angina pectoris for three years before I^{131}. He received 27 and 22 millicuries of I^{131} on June 7 and June 15, 1949, respectively. The 24-hour thyroid uptakes were 40 and 9 per cent, respectively. A radiation dose of approximately 40,500 REP was delivered to the thyroid gland. No thyroiditis was noted. Dramatic relief of angina accompanied the development of hypothyroidism; symptoms of the latter were controlled by 6–18 mg. of thyroid daily. Eighteen months after I^{131} therapy, a mild recurrence of anginal symptoms was experienced. Angina became progressively more severe during the following six months and culminated in an acute myocardial infarction. The patient expired on November 14, 1951, eight hundred and ninety days after the administration of I^{131}.

Postmortem examination

Cardiac pathologic diagnoses were: 1) coronary atherosclerosis with old occlusion of the right coronary artery, 2) healed myocardial infarction, and 3) miliary fresh infarcts of the left ventricle and interventricular septum.

Thyroid and neck structures: The thyroid gland weighed 5.6 grams. It was white and fibrous, but of normal configuration. The right lobe measured $3.5 \times 1.7 \times 1.0$ cm. and the left, $3.0 \times 1.5 \times 1.2$ cm. The left lobe contained a small cavity filled with red fluid. The larynx and trachea were normal.

The normal thyroid architecture had been replaced by a mesh of intersecting heavy, hyalinized, dense fibrous bands, which enclosed islands of parenchyma. There were isolated cells and masses of poorly staining colloid in the midst of dense connective tissue. Some cells were round or oval and stained clear; other cells were pyknotic with unusually large irregularly shaped and hyperchromatic nuclei, often in close relation to masses of colloid. Other areas were characterized by progressive follicular destruction with desquamation of the lining epithelium, disruption of the follicular wall and ex-

trusion of poorly staining colloid. Scattered in the stroma were groups of relatively normal acini with evenly staining eosinophilic colloid in follicles lined with flat epithelium. The vessels showed considerable hyalinization of the intima. There was very little inflammatory cell infiltration.

Adrenals: Normal in size, shape and color. No histologic abnormality was noted.

Parathyroids: Two parathyroids were found; they were normal in size, shape, color and consistency. On histologic examination the most common cell was the chief cell. Nests of oxyphil cells were seen. The fibrous stroma was somewhat increased and the vessel walls were slightly thickened. No evidence of infiltration or tumor was found.

Pituitary: The pituitary, somewhat oblong, measured $1.3 \times 0.3 \times 0.3$ cm. and was firm. The cut surface was homogeneous, white and granular. On microscopic examination there was some increase in the number of basophils. A small, partially encapsulated collection of chromophobe cells was noted. Some possessed large, irregular nuclei. There was no other evidence of tumor, fibrosis or necrosis.

Comment: The most notable findings in this case, studied for eight hundred and sixty-nine days after I^{131} administration, were not only the fibrosis of the thyroid gland but the evidence of a continuing process of thyroid degeneration. In the areas of continuing degeneration, atypical bizarre cells with large nuclei, hyperchromatic and vacuolated, were observed. As in Case 13, it is likely that this was due to progressive diminution of blood supply and the effects of increasing fibrosis. It is striking that this patient manifested few symptoms of the hypometabolic state of myxedema while receiving 18 mg. of desiccated thyroid daily.

Case 16. M. G., BIH #24430, a 49-year-old man had hypertension for nine years before, myocardial infarctions seven and five years before, and chronic congestive heart failure one and a half years before I^{131} therapy. He received 27.0 and 30.5 millicuries of I^{131}, respectively, on October 12 and October 23, 1948. Twenty eight per cent and 36 per cent, respectively, were excreted in the urine during the following seventy-two hours. Striking improvement of congestive failure accompanied the development of hypothyroidism; symptoms of the latter were controlled by 18 mg. of desiccated thyroid daily. Three years after I^{131} therapy, angina pectoris developed and culminated in an acute myocardial infarction. He expired one thousand and ninety-two days after the administration of I^{131}.

Postmortem examination

Pathologic diagnoses were: coronary atherosclerosis, multiple healed myocardial infarcts, ischemic necrosis of the myocardium, cardiac hypertrophy and dilatation, pleural and pericardial effusions, pulmonary edema, and cirrhosis of the liver.

Thyroid: The thyroid, small and atrophic, was buried in dense connective tissue. It measured $2.2 \times 3.5 \times 0.5$ cm. and weighed 4.7 grams; it was firm and of a light, red-brown color. There were no nodules or tumors.

Multiple sections from two areas were examined histologically. The major part of the thyroid had been replaced by sheets of dense acellular connective tissue. At the periphery of the gland, in the subcapsular area, remnants of thyroid tissue were found. In some areas these consisted of cells of irregular shape, size and staining, near shadows or follicles which no longer contained any colloid or near isolated masses of eosinophilic-staining colloid without any epithelial border. Some cells had nuclei which were very large and hyperchromatic. Mitoses were absent. In other areas, islands of thyroid tissue were demarcated into lobules by collagenous bundles. Within these lobules were groups of follicles, none normal, in various states of disintegration. A few contained deeply

staining eosinophilic colloid; in others, the colloid was pale and fragmented; in most, there was no colloid, but an eosinophilic cellular debris. The epithelium, where present, was often shrunken and deeply staining, with bizarre nuclei. The arteries showed intimal thickening and hyalinization.

Neck structures: The trachea, larynx, vocal cords, and cartilages were normal. There was no evidence of inflammation, stenosis or tumor.

Parathyroids: Three parathyroids were examined histologically. All contained a moderate amount of fat. The most common cell was the chief cell, but islands of water-clear cells were noted. There was no evidence of necrosis, fibrosis, or tumor.

Adrenals: Some scarring in relation to a previous nephrectomy was noted. Otherwise, the zoning was distinct, and the glands were well filled with lipid. No evidence of necrosis or tumor was found.

Pituitary: The gland weighed 1.3 Gm. and appear normal grossly. On histologic examination there was slight capsular thickening. Two small basophilic adenomata were noted. The pars intermedia contained many cystic follicles filled with dense colloid and lined by flattened cells. No evidence of fibrosis, necrosis or other tumor was noted.

Comment: The replacement of most of the thyroid gland by dense collagenous tissue was the major finding in this case. Notable, also, was the evidence at the periphery of some residual thyroid tissue still undergoing progressive degeneration one thousand and ninety-two days after I^{131} therapy. In this area, cells of bizarre characteristics were noted. In this case, as in Case 15, there was persistent hypometabolism beginning three and a half months after administration of I^{131} and persisting for thirty-two months, until death. There were few symptoms of myxedema during treatment with 18 mg. of desiccated thyroid daily.

THE PATHOLOGIC PICTURE OF I^{131} RADIATION IN THE EUTHYROID HUMAN

The pathologic findings and radiation data in the present series of 16 cases are summarized in Table 1. The pathologic data are based on routine hematoxylin and eosin stained sections prepared in the Department of Pathology, Beth Israel Hospital.

Seven days after the oral administration of 17 and 20 millicuries (Cases 1 and 2), estimated to have delivered 14,500 and 31,000 REP, respectively, to the thyroid gland, no histologic changes (Fig. 1) were noted which could be attributed to I^{131}. Data previously published (5) indicate that at a comparable interval after equivalent I^{131} radiation, there was marked impairment in the ability of the thyroid gland to take up and retain radioiodine. Fourteen and twenty-four days after 59 and 26.4 millicuries (Cases 3 and 4), delivering approximately 105,000 and 75,000 REP, respectively, massive radiation effects occurred. There was extensive distortion and destruction of the normal thyroid architecture, maximal at the center of the gland. In this area, only shadows of follicles remained, the follicles having been replaced by masses of eosinophilic-staining fibrinoid material or by dense infiltration with polymorphonuclear leuckoytes (Fig. 2). The stroma was edematous and showed hyaline degeneration. The blood vessels showed marked acute changes including fibrinoid degeneration of the

intima (Fig. 3). Small vessels were ruptured and hemorrhage into the follicles and stroma was common. At the periphery of the gland, the destruction was less marked and the follicular pattern was maintained. However, the lumen was usually occupied by a vacuolated fragmented colloid admixed with cells desquamated from a broken epithelial border. Infiltration, although less intense than at the center, was still notable.

The picture of marked acute pathologic change was not restricted to the first few weeks but was noted even eleven weeks after administration of I^{131}. In Case 7, studied eighty days after 43 millicuries of I^{131}, the thyroid presented a histologic appearance very similar to that in Cases 3 and 4,

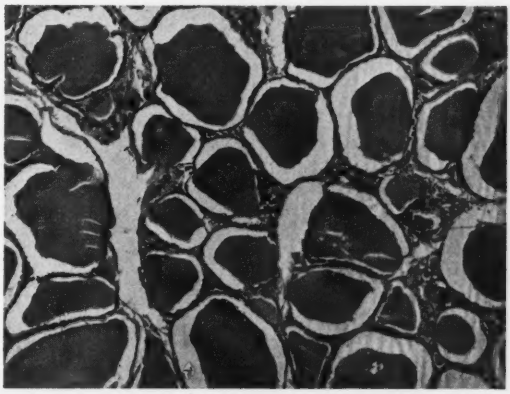

FIG. 1. Case 2—Normal thyroid gland seven days after administration of 20 millicuries of I^{131} delivering 31,000 REP to the thyroid gland (×100).

with the indistinct eosinophilic appearance of infarcted tissue, loss of follicular architecture, polymorphonuclear infiltration and vascular damage (Fig. 4). It is unlikely that this was solely a direct effect of radiation on thyroid parenchyma, but rather that it resulted also from marked vascular injury with multiple occlusions due to radiation.

In Cases 5, 6, 8, 9, and 10, in which the radiation delivered was less intense or the interval from I^{131} therapy to necropsy was greater than in Cases 3, 4 and 7, alterations of thyroid structure were less acute. The architectural pattern was maintained in a stroma of increased fibrous tissue (Figs. 5 and 6). Infiltration was less intense and consisted largely of lymphocytes. Small arteries showed intimal thickening and hyalinization. The lumen of the follicles was occupied by desquamated epithelial cells which often assumed bizarre forms, and by a golden yellow pigment (Figs. 5 and 6). Large atypical hyperchromatic cells, multinucleated or with vacuolated nuclei, were seen in the fibrous tissue frequently in association

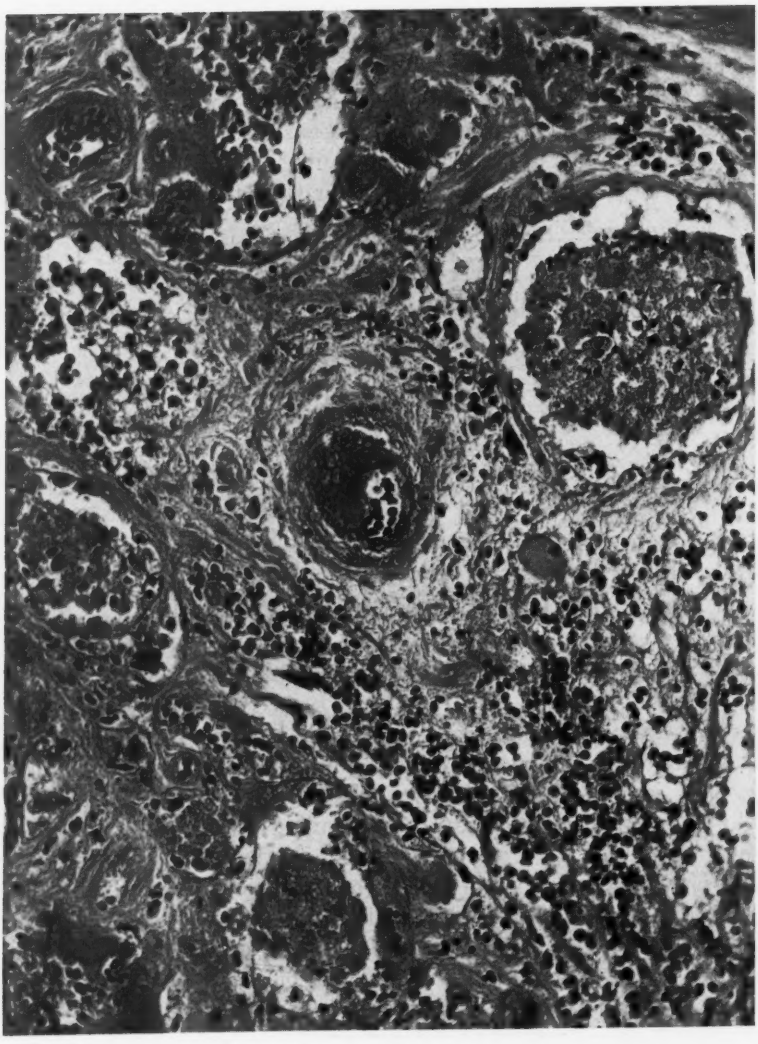

FIG. 2. Case 3—The pathologic changes observed in the thyroid fourteen days after administration of 59 millicuries of I^{131}. The central arteriole shows intimal fibrinoid degeneration. There is disruption of follicles and polymorphonuclear infiltration (×400).

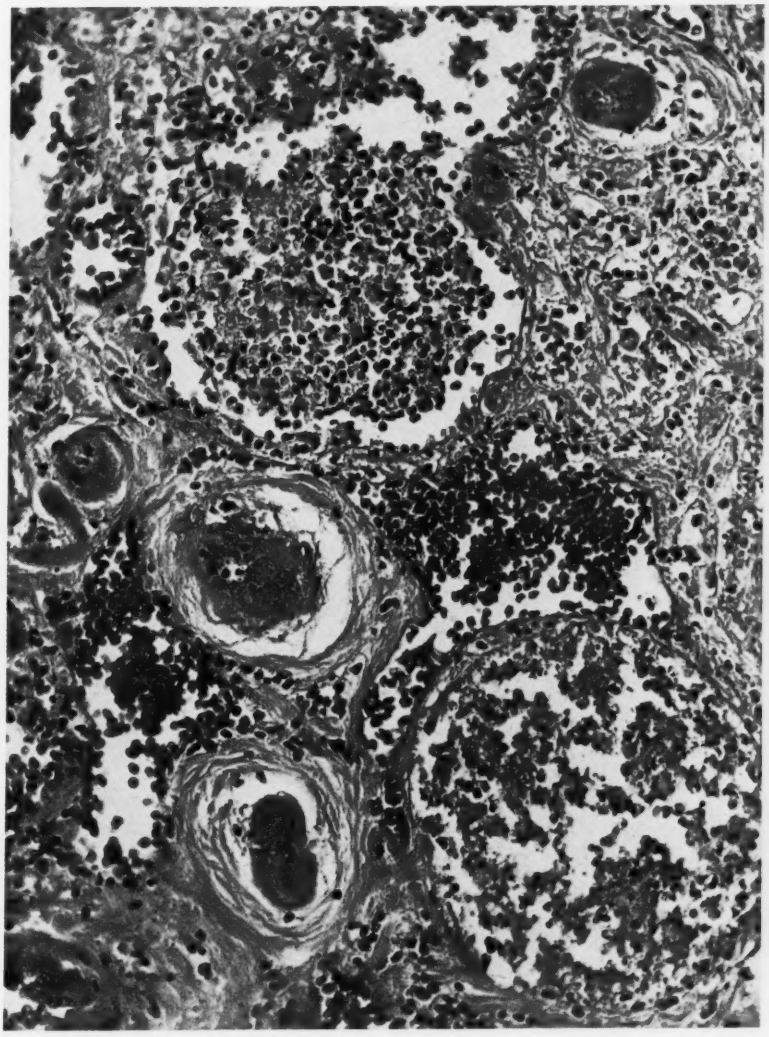

FIG. 3. Case 3—Marked vascular change, including obliteration of the lumen, polymorphonuclear infiltration, and rupture and hemorrhage into follicles (×400).

FIG. 4. Case 7—The pathologic changes in the thyroid gland eleven weeks after administration of 43 millicuries of I^{131} (×100).

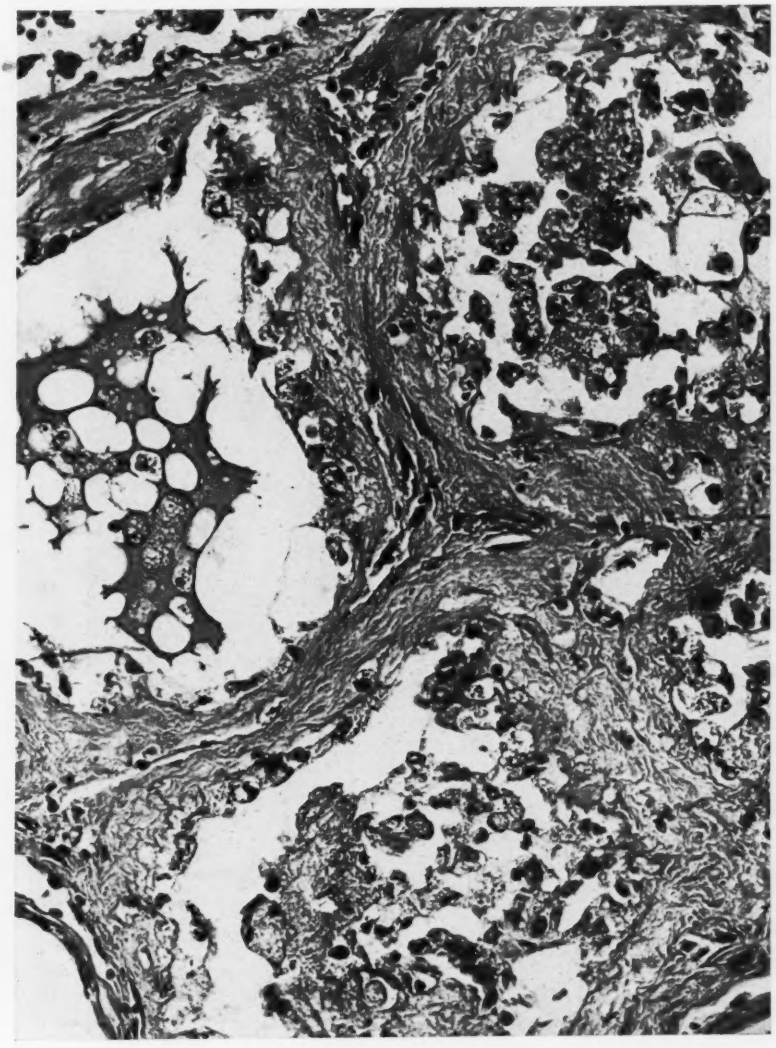

FIG. 5. Case 5—The pathologic changes in the thyroid resulting from 66.5 millicuries of I^{131} administered during the five weeks before death. (*See* text for description) (×500).

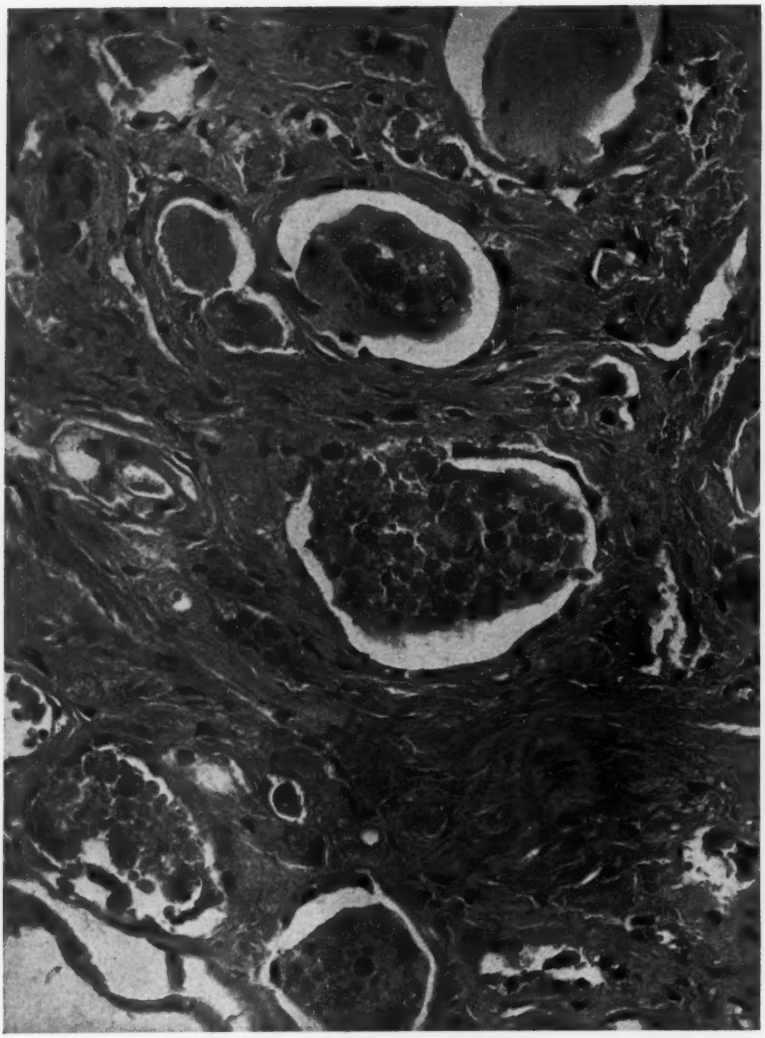

FIG. 6. Case 10—The pathologic changes in the thyroid following 45 millicuries of I^{131} given from 42 to 186 days before death. There is marked fibrosis, fragmentation and alteration of colloid (×400).

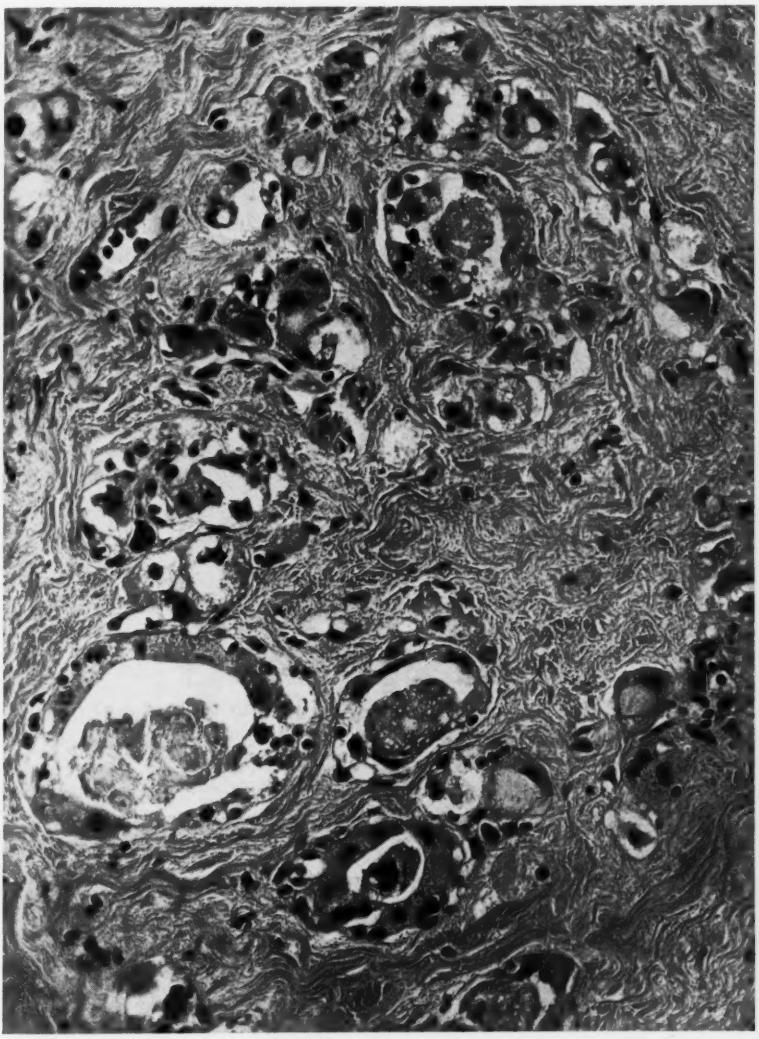

FIG. 7. Case 15—The pathologic changes in the thyroid 890 days after administration of 49.5 millicuries of I^{131}. There is evidence of progressive follicular degeneration. Many large, atypical cells with hyperchromatic nuclei are noted (×400).

with masses of colloid no longer in follicles (Fig. 6). The colloid which remained within the follicles was abnormally fragmented, in globules or irregular stringy masses which stained poorly and were often admixed with cells (Figs. 5 and 6).

These subacute or chronic changes were apparently progressive over a long period. In Case 15, examined eight hundred and ninety days after I^{131} therapy a wide range of radiation pathology was visible, with many follicles at various stages of disintegration and isolated epithelial cells buried in an increased stroma (Fig. 7). Since 97 per cent of the radiation from I^{131} is delivered in forty days after administration, such long-term progressive changes can most likely be ascribed to the constricting effect of increasing fibrosis or to progressive decrease in blood supply associated

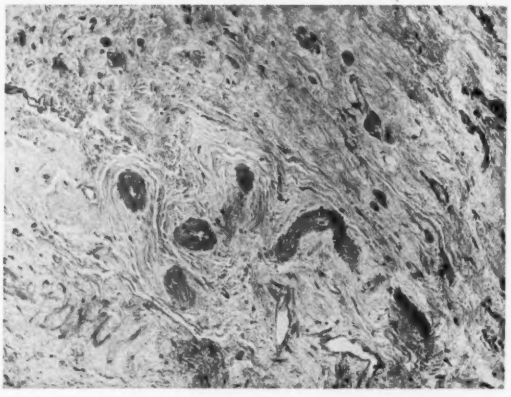

FIG. 8. Case 14—The late stages of changes in the thyroid 869 days after administration of I^{131}. There is marked fibrosis and vascular thickening, with narrowing of the lumen (×100).

with the arterial narrowing produced by subintimal and intimal thickening.

The late tissue alterations due to radiation were best seen in Cases 11–16, in which the patients survived over three hundred days after I^{131} administration. The thyroid glands of some of these patients also demonstrated subacute changes, as just described. The late stages of I^{131} radiation in the thyroid consisted in a dense, largely avascular, collagenous eosinophilic connective tissue. The vessels had a hyalinized and greatly thickened intima (Fig. 8). In these sections, which could hardly be recognized as thyroid, there were scattered in the connective tissue occasional cells or groups of cells similar to those described in the subacute phase, with large, pleomorphic, hyperchromatic nuclei and prominent nucleoli,

usually not in association with any colloid. There was little or no infiltration. This markedly thickened connective tissue and its associated changes were also visible to a lesser degree in most of the earlier cases, *e.g.*, Cases 6, 9 and 10. In these latter instances, relatively normal follicles were visible in the same sections in the dense fibrous tissue.

Nodules: The irregular distribution of I^{131} in the thyroid (18) and the presence of nonfunctioning thyroid nodules has been previously described (19). In view of the short tissue range of the beta ray of I^{131}, it is not surprising, therefore, to note the resistance of nodular tissue to radiation effects. In Case 9, the thyroid gland one hundred and twenty-five days after I^{131} therapy consisted of a relatively intact colloid nodule in a mass of dense connective tissue and a few residual follicles (Fig. 9). Clinical ex-

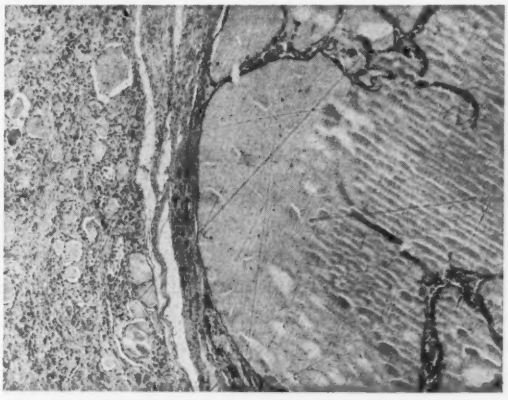

FIG. 9. Case 9—The pathologic changes following administration of 35.5 millicuries of I^{131}, showing an intact colloid nodule in a dense fibrous stroma (×100).

perience has indicated that permanent hypometabolism does not readily develop in such instances. Repeated doses of I^{131} are required. After such therapy, however, we have observed the disappearance of nontoxic nodules which were previously readily palpable. It was logical to assume, therefore, that following the destruction of all normal thyroid tissue, the nonfunctioning nodule, previously protected from radiation effects by its inability to take up I^{131}, assumed function and became metabolically active. Evidence for this was observed in Case 8. The thyroid gland in this case was extremely firm and contained multiple nodules in both lobes, separated by firm fibrous tissue. Microscopically the parenchyma was replaced by degenerating follicles in a dense fibrous tissue. In one of the many colloid nodules, found in the midst of the fibrosed residue of the thyroid gland, a wedge-shaped area of acute damage was noted (Fig. 10). The evidence of

FIG. 10. Case 8—Radiation injury observed in a thyroid nodule (×100).

acute damage in this nodule was ascribed to the effect of the second dose of 21 millicuries of I^{131}, thirty-one days before death.

THE RELATION OF THE CLINICAL STATE TO THE PATHOLOGIC PICTURE

The clinical effects of the administration of I^{131} to euthyroid cardiac patients have previously been described by us (4). The present data permit correlation of the metabolic state with the pathologic picture observed at necropsy. In Cases 1–8, no evidence of hypometabolism was noted. In Cases 1 and 2 there was no evidence clinically or at necropsy of I^{131} thyroid radiation damage. In Cases 3, 4, 5 and 7 insufficient time had elapsed to permit the development of hypometabolism. The thyroid glands in these cases were the site of intense acute irradiation reaction and hypometabolism might well have developed subsequently. In Case 6, the effect of radiation had apparently come to an end. Normal follicles were embedded in an increased fibrous stroma. No acute reaction was seen. It is unlikely that hypometabolism would have developed in this case without additional I^{131}. In Cases 8 and 9, although the normal thyroid parenchyma had been destroyed, the well filled colloid nodules apparently had sufficient function to maintain a euthyroid state. Transient hypometabolism developed in Patient 10; as noted previously, the transient nature of the hypothyroidism in this patient can be explained by the persistence of function in residual thyroid follicles (Fig. 11). Permanent hypometabolism developed in Cases 11–16. In these instances there was a rough correlation between the amount of residual thyroid tissue and the degree of hypometabolism. In Cases 12 and 14 scarcely a single colloid-containing follicle could be recognized (Fig. 8) and the patients were grossly myxedematous without thyroid medication. In Cases 15 and 16 hypometabolism

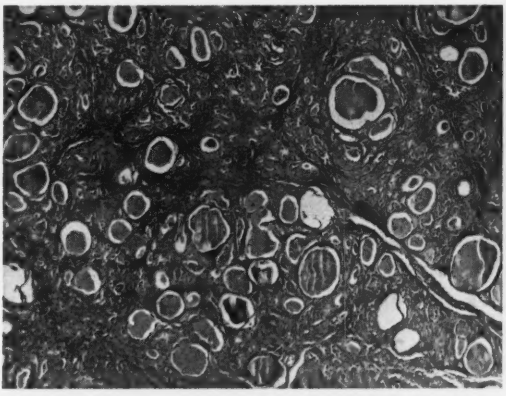

FIG. 11. Case 10—The persistence of residual thyroid follicles in a dense fibrous stroma (×100).

developed slowly and gradually, and for a long period it was asymptomatic. In these cases some residual follicles were found which apparently maintained at least a low level of hormonal activity.

REGENERATION AND NEOPLASIA

The nature of the large atypical cells (Fig. 7) with hyperchromatic nuclei, seen long after radiation, remains obscure. Observations over a longer period are necessary to determine whether they represent an effort to regenerate or whether they are possibly neoplastic. Anaplastic nonencapsulated thyroid neoplasms have been described in the rat eighteen months after the administration of 400 microcuries of I^{131} (20). We have found no evidence of such neoplasia in our series; the longest period of follow-up was one thousand and ninety-two days after the administration of 57.5 millicuries (Case 16). In Case 8, large eosinophilic cells resembling Hürthle cells were seen in a relatively intact adenoma. Their presence probably antedated I^{131} therapy.

EFFECT OF I^{131} RADIATION ON OTHER ORGANS

Studies in mice and rats have indicated damage to the tracheal epithelium, the parathyroids and the recurrent laryngeal nerve following large doses of I^{131} (12, 15). We have not found evidence of radiation injury to these structures despite specific search. In several instances, parathyroid glands have been buried in the substance of a thyroid which showed evidence of extensive destruction and fibrosis from I^{131} (Fig. 12). The parathyroids, however, were intact in all but one of these cases. In Case 7, a fragment of parathyroid was found embedded in a thyroid gland showing

evidence of extreme irradiation damage (Fig. 4). The oxyphil cells of the parathyroid gland showed slight swelling and vacuolization. Clinical evidence of parathyroid functional impairment has not been observed (21).

Gorbman has described the development of large noninvasive chromophobe tumors of the anterior pituitary in more than 90 per cent of mice eight months after administration of I^{131}. Nine pituitary glands have been examined in our series. Enlargement of the pituitary was noted in 1 case (Case 13) but microscopic study showed no abnormalities. Patient 10 showed extensive fibrosis of the anterior pituitary after three doses of I^{131} administered 184, 140 and 42 days, respectively, before death. This patient, however, had suffered several episodes of profound shock before death and the pituitary alterations may have been consequent to these. The pituitary glands of 2 patients (Cases 14 and 16) showed minute basophilic adenomata; and several other patients showed increased numbers of basophils in the pituitary. A localized small, noninvasive, nonencapsulated collection of chromophobe cells was observed in Case 15.

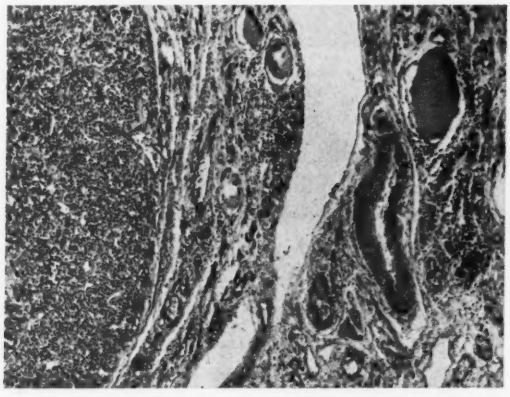

FIG. 12. Case 8—A normal parathyroid adherent to a thyroid gland showing marked fibrosis following administration of I^{131} (×100).

SUMMARY AND CONCLUSIONS

1. The pathologic findings are described in 16 euthyroid patients who expired from seven to one thousand and sixty-nine days after the oral administration of from 17 to 157 millicuries of I^{131}. Radioactive iodine was utilized in 15 of the 16 patients because of intractable angina pectoris or congestive heart failure; the remaining patient had a primary carcinoma of the thyroid.

2. Seven days after administration of 17 and 20 millicuries of I^{131}, which

delivered 14,500 and 31,000 REP, respectively, to the thyroid gland, no histologic changes were noted which could be attributed to I^{131}.

3. Fourteen and twenty-four days, respectively, after administration of 59 and 26 millicuries of I^{131}, marked central destruction of the thyroid gland was noted. There was edema and degeneration of the stroma, striking acute vasculitis with thrombosis and hemorrhage, epithelial swelling and vacuolization, follicular destruction and polymorphonuclear infiltration. At the periphery of the thyroid gland radiation damage was less severe, but there was extensive disruption of follicles and colloid and round-cell infiltration.

4. The thyroid gland of patients who survived a longer period showed increased fibrous stroma, lymphocytic infiltration, arteriolar intimal thickening and hyalinization. The follicular epithelium was desquamated and admixed with a fragmented or globular colloid. Atypical cells with large hyperchromatic nuclei were noted.

5. In patients who survived for three hundred and sixteen to one thousand and sixty-nine days after treatment with I^{131}, the thyroid gland was largely replaced by dense fibrous tissue. The small arteries were notably thickened. The remaining follicles were markedly disrupted, with fragmentation of colloid and destruction and desquamation of the follicular epithelium. Atypical cells with large hyperchromatic nuclei were noted; mitoses were absent.

6. Continued degeneration of thyroid follicular epithelium was present long after the administration of I^{131}. The relative role of progressive vascular damage and increasing fibrosis in this process are discussed.

7. The parathyroid glands were examined in 9 cases. There was no evidence of abnormality except in Case 7 (ten weeks after administration of I^{131}). In this instance there was swelling and vacuolization of oxyphil cells in a fragment of parathyroid embedded within the thyroid gland.

8. Fibrosis of the pituitary was seen in 1 case but could not be clearly attributed to I^{131}. In the other pituitary glands examined, no histologic changes were noted which could be ascribed to I^{131}.

9. No radiation injury to the trachea, larynx, or adrenal was found.

10. No thyroid neoplasm was found which could be attributed to I^{131}.

Acknowledgment

We are indebted to Dr. William A. Meissner of the New England Deaconess Hospital, who reviewed the thyroid sections.

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THERAPEUTIC EFFECTS FROM REPEATED DIAGNOSTIC DOSES OF I^{131} IN ADULT AND JUVENILE HYPERTHYROIDISM*†

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TRACER methods with small doses of radioiodine, I^{131}, are commonly used in hyperthyroidism to follow the course of the disease under the influence of a particular therapy. It is generally assumed that these small amounts of isotope in themselves have no physiologic effect. Evidence obtained in this clinic from three directions has indicated that this assumption may not be correct. First, it has been shown that some patients with hyperthyroidism are unusually sensitive to radiation effect from therapeutic doses of I^{131} (1). This suggests that smaller doses of isotope also may evoke a response. Second, it has been found in one clinic that an occasional remission from thyrotoxicosis may follow ACTH administration (2, 3), whereas this response has not been noted in another clinic (4). The only apparent difference which can be demonstrated between the two investigations is in the use of diagnostic tracer methods with I^{131}. Larger and more frequently repeated doses of I^{131} have been administered in the former compared to the latter. Third, it has been a source of some speculation as to why occasional patients with hyperthyroidism claim to feel better after a single diagnostic tracer test. It is questionable whether a factor of suggestion is the only reason.

Accordingly, the present investigation was undertaken to observe the effect on hyperthyroidism of the administration of several repeated diagnostic doses of I^{131} in the absence of other therapy. Tracer doses of I^{131} were administered in about the same total number and individual dosage as ordinarily used in investigative studies. As stated, no other therapy was allowed. Definite physiologic effects can be shown to have resulted from

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the several tracer tests alone, to the extent of complete remission of hyperthyroidism. Hyperthyroid children, especially, have appeared responsive to the diagnostic doses of I^{131}.

METHODS

The same laboratory (5) and clinical (6) procedures employed in previous work have been followed in the present study. Only patients with unquestioned toxic diffuse or recurrent goiter have been treated. Radioiodine, I^{131}, from Oak Ridge was administered without carrier, according to two dosage schedules: 40 microcuries weekly for five to six doses, or 100 to 200 microcuries weekly for three doses. In many patients the initial series of doses of I^{131} was repeated after several weeks, but in a few the second series consisted of larger doses.

TABLE 1. TOTAL DOSAGE AND ULTIMATE RESULT FROM SEVERAL REPEATED TRACER DOSES OF I^{131} IN 29 PATIENTS WITH TOXIC DIFFUSE AND TOXIC RECURRENT GOITER

Total dose of I^{131} (μc.)	Remissions		
	Complete	Partial	None
150–199			1
200–249	4	1	5
250–299	1		2
300–499			2
500–699	1		4
700–899	2*		3
900–1,099		2	1

* These 2 patients had a sharp decline in 24-hour uptake of I^{131} after administration of 400 and 500 μc. of I^{131}, respectively.

RESULTS

There were 29 patients who received repeated diagnostic doses of I^{131}. There were 2 men, 21 women and 6 children. Adult ages ranged from 27 to 64, and the children were from 7 to 12 years old. Individual and total dosages of I^{131}, and the results in terms of remission from hyperthyroidism are listed in Table 1. It is seen that of the entire group, 8 of the patients experienced complete remission of hyperthyroidism, 3 partial recovery, and 18 no improvement. Of the 6 children, 4 achieved complete remission. The minimum dosage of I^{131} resulting in complete remission was 200 to 240 μc. This provided approximately 27 to 100 roentgens equivalent physical (r.e.p.) to the thyroid gland per 40-μc. dose of I^{131}, and a total radiation of about 161–607 r.e.p. (Tables 2 and 3). It should be pointed out that the 24-hour uptake of radioiodine by the thyroid in 2 of the pa-

tients entering a period of complete remission and recorded as having received 800 or 900 μc., had fallen sharply after 400 and 500 μc. of I^{131}, respectively, had been administered. Laboratory values for the 8 patients entering a period of complete remission are shown in Table 3. Only 2 of these patients remained euthyroid beyond three months; the rest had a recurrence of hyperthyroidism.

TABLE 2. AMOUNT OF RADIATION RECEIVED BY THE THYROID GLANDS OF PATIENTS ENTERING A PERIOD OF COMPLETE REMISSION OF HYPERTHYROIDISM AFTER REPEATED TRACER DOSES OF I^{131}

Patient	I^{131} dosage schedule (μc)	Total I^{131} given (μc)	Radiation received from	
			Single dose of I^{131} (r.e.p.)*	Total I^{131} dosage (r.e.p.)*
J.R.	40×6	220	32	194
S.C.	40×6	240	100	607
D.P.	40×6	240	27	161
	40×7†	280	29	202
J.K.	40×6	240	89	532
R.A.	40×6	260	41	266
H.C.	100×3	300	62	187
	100×3‡	300	77	231
L.F.	100×3	300	37	110
	100×5‡	480	37	185
R.S.	100×5	500	82	410
	100×4§	440	79	324

* Roentgen equivalents physical.
† Six months between series.
‡ Three weeks between series.
§ Three months between series.

Significant changes in single laboratory values are noted in many of the patients failing to respond otherwise to the I^{131} (Table 4). A fall in basal metabolic rate of 15 per cent or more, a decrease in serum precipitable-iodine level of 2.0 gamma per cent or more, and a decrease in 24-hour thyroidal uptake of I^{131} of 15 per cent or greater, have been defined as significant.

TABLE 3. RESULTS OF LABORATORY TESTS FOR THYROID FUNCTION, BEFORE AND FOLLOWING REPEATED TRACER DOSES OF I^{131} IN PATIENTS ENTERING A PERIOD OF COMPLETE REMISSION OF HYPERTHYROIDISM

Patient	Total radiation received	Laboratory values					
		BMR		24-hr. I^{131} uptake		SPI*	
		Before	After	Before	After	Before	After
	r.e.p.	%	%	%	%	γ%	γ%
D.P.	161	+35	−31	68	7	12.6	4.9
J.R.	194	+18	−8	58	36	13.4	16.4
R.A.	266	+6	−16	64	20	8.8	5.5
L.F.	295	+80	−6	66	4	20.4	6.0
H.C.	418	+31	−3	58	37	12.6	8.6
J.K.	532	−2	−34	62	20	9.6	5.4
S.C.	607	+19	+6	45	23	10.4	3.7
R.S.	734	+25	−11	66	6	14.0	4—8

* Serum precipitable iodine.

TABLE 4. INITIAL AND LOWEST LABORATORY VALUES FOR TESTS, SHOWING SIGNIFICANT DECLINE FOLLOWING REPEATED TRACER DOSES OF I^{131} IN HYPERTHYROID PATIENTS OTHERWISE FAILING TO RESPOND

Patient	Laboratory values					
	BMR		24-hr. I^{131} uptake		SPI*	
	Before	Low	Before	Low	Before	Low
	%	%	%	%	γ%	γ%
E.S.	+30	+14				
M.V.	+32	+6				
F.B.	+54	+20	70	54		
T.C.	+50	+23	72	58		
L.D.			71	48		
C.J.			64	44		
H.L.			76	52		
A.T.		*M*	48	33		
B.G.					11.7	9.1
E.R.					13.1	10.5
D.P.					13.5	9.4

* Serum precipitable iodine.

DISCUSSIONS AND CONCLUSIONS

The preceding findings reveal that repeated 40 μc. to 100 μc. diagnostic doses of I^{131}, totaling between 200 and 700 μc., have significant therapeutic effects in some hyperthyroid patients. As a consequence, the conclusions from experiments in which diagnostic tracer tests with I^{131} have been used to follow the course of hyperthyroidism under the influence of some agent or procedure, must be re-evaluated in order to take into account the I^{131} administered. The occasional remission from hyperthyroidism with ACTH or with cortisone, reported by the Boston investigators (2, 3) and not obtained by this clinic (4), thus may have to be re-examined in the light of the present study. The use of larger individual doses of I^{131} and the more frequent repetition of these doses by the Boston group may well be the true reason for instances of recovery from hyperthyroidism, and not the the hormonal therapy.

The radiation to the thyroid from a single diagnostic dose of I^{131} as given to the patients entering a period of remission of hyperthyroidsm in this study, has been approximately 30 to 100 r.e.p. The total radiation provided to the thyroid of these patients has been 780 r.e.p. or less. This is in contrast to the level of about 4,000 to 6,000 r.e.p. generally considered the minimum necessary for a successful outcome from therapeutic doses of I^{131} (1). Although the remissions in this study were almost all temporary, it is indicated that the present lower limits of dosage with I^{131} for therapeutic purposes may be reduced in certain selected patients. The need for a method to distinguish such patients with radiosensitive glands is re-emphasized.

By the same token, it would appear that a single dose of I^{131} may produce transient inhibition of the hyperthyroid gland from a radiation effect of 30 to 100 r.e.p., if reports of well-being after one such test can be interpreted on this basis. An organic explanation, at least in some of these patients, appears as reasonable as a psychologic one.

It should be emphasized that children with hyperthyroidism appear to respond fairly consistently to the low levels of radiation effect from I^{131} provided in this study. The fear of late radiation cancer probably will serve to prevent this finding being put to therapeutic use. However, the use of low levels of external radiation in this age group deserves to be fully explored. A favorable result would provide a simple and psychologically nontraumatic method of therapy for this age group. The epiphyses of the cervical vertebrae may be injured by radiation effect and obviously need to be safeguarded in such an investigation.

The present results also stress the need to decrease the amount of I^{131} used in diagnostic tests for routine and investigative purposes. Although

it is probable that the euthyroid gland, unlike the hyperthyroid one, is not affected by a single dose of from 40 to 100 μc. of I^{131}, the widening use of tracer methods increases the likelihood that the tests may be repeated in a second or third clinic. It thus becomes urgent to use the smallest practicable dose of I^{131}, in order to prevent piling up of radiation effect.

The possibility that the remissions obtained in the present study may have been spontaneous and independent of radiation effect from I^{131} must be considered. Lerman (7) estimates that in 1 of 25 patients with hyperthyroidism a remission occurs spontaneously. This figure is considerably less than the 1 in 4 remission incidence of the present study. Spontaneous remissions also are generally long sustained. In only 2 of 8 patients having a remission from repeated diagnostic doses of I^{131} was euthyroidism maintained beyond three months. The incidence of spontaneous remission during the first three months after the initial visit to the clinic for treatment of hyperthyroidism is much less than the over-all figure of 1 in 25. On the other hand, in half the patients in the present study remission occurred by six to eight weeks after initiation of treatment with I^{131}. This time interval is the same as that required for therapeutic doses of I^{131} to induce euthyroidism. More than that, the time intervals during which remission and, later, recurrence took place were reduplicated when a second series of identical doses of I^{131} was given to one of the patients in this study.

Improvement, as indicated by changes in individual laboratory values in some of the patients otherwise unaffected by repeated doses of I^{131}, is difficult to explain. The disruption of correlation between uptake of iodine and hormonal release, which has been noted after treatment of hyperthyroidism (8), may possibly account for the lack of parallelism between decreases in I^{131} uptake and in serum precipitable iodine level. A time factor may also be involved. The lack of correlation between the decreases in basal metabolic rate and in serum precipitable iodine level found in these patients may depend on a time factor or on the relative crudeness of both the basal metabolic rate and serum precipitable iodine determinations. Finally, the fluctuation in laboratory values may be spontaneous and have nothing to do with the effect of isotope administration, although this appears less likely. At any rate, the occasional finding of greatly discrepant results from the various laboratory tests of thyroid function in untreated hyperthyroidism appears to have been reproduced by the present study.

SUMMARY

1. Repeated diagnostic doses of I^{131} have been given to 29 hyperthyroid patients in the same total amount of individual dosage as that generally

given in order to follow the course of hyperthyroidism during investigative studies.

2. Various changes in thyroid activity have been obtained, to the extent of complete remission of hyperthyroidism in 8 patients. Remission generally has not lasted more than three months.

3. In children, the hyperthyroid gland appears especially responsive to low levels of radiation. The therapeutic possibilities of external radiation are suggested.

4. The conclusions from investigations in hyperthyroidism, in which several diagnostic doses of I^{131} have been given, need reappraisal to rule out the effect on the hyperthyroidism of radiation provided by these diagnostic tracer procedures.

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OBSERVATIONS ON THE NODULAR THYROID GLAND WITH THE GAMMAGRAPH*

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INTRODUCTION

THE introduction of the scintillation counter (1, 2, 3, 4) to clinical medicine, as a possible replacement for the less-sensitive gamma-detecting Geiger-Mueller counter for *in vivo* studies in human beings, has led to many interesting observations and has suggested several new applications of radiotracers. Because of the increased efficiency of the scintillation counter for gamma radiation, it became possible to construct a specially designed gamma-detecting scintillation counter with an optical geometry whose resolution is such that the isotope-concentrating tissue can be outlined in such a manner that a silhouette indicating the size and shape of the tissue can readily be obtained. The silhouette of the isotope-concentrating tissue, for the sake of simplicity and clarity, has been designated a gammagram, so named because the principle of its production is similar to the principle involved in making a photograph. Instead of an image created by photons of visible light, a gammagram represents an image created by photons of gamma rays as recorded by means of an automatic scanning device, utilizing a calcium tungstate scintillation counter as a detector or "camera." Thus it is possible to "photograph" or gammagraph any gamma-emitting, isotope-concentrating tissue located beneath the surface of the body. The automatic scanner or gammagraph used in these studies represents a refinement of the manual scanner presented in 1950 (5).

The first clinical application of this instrument was for outlining the diffuse hyperplastic thyroid gland as a means of determining its weight, which is essential for the proper calculation of dosage and proper evaluation of radioiodine therapy used for the treatment of thyrotoxicosis. With the gammagraph it has been possible to determine the weight of the

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thyroid gland within 10 per cent of its actual weight. As an outgrowth of this study and because of the increased sensitivity and excellent resolving power obtainable with the scintillation counter, it has become possible to differentiate variations in the degrees of radiation activity within the parenchyma of normal and abnormal thyroid glands. It is the purpose of this paper to present further observations in the study of the nodular thyroid gland, using the gammagraph.

METHODS

The design and physical characteristics of the automatic scanner and "directional" scintillation counter used in these studies, as well as the initial clinical application of this instrument in the study of the nodular and of diffusely enlarged thyroid gland, have been described elsewhere (6, 7). Recently a similar instrument has been reported (8).

The aperture of the distal end of the tube is $\frac{1}{4}$ inch in diameter surrounded by a $\frac{3}{4}$-inch tungsten shield, 1 inch in length, thus providing good collimation. With this tube 1 microcurie of radioactive iodine has a net counting rate of 10 counts per second. When comparison with the Geiger-Mueller tube under comparable geometric conditions is

FIG. 1. The gammagraph is shown in position over the subject.

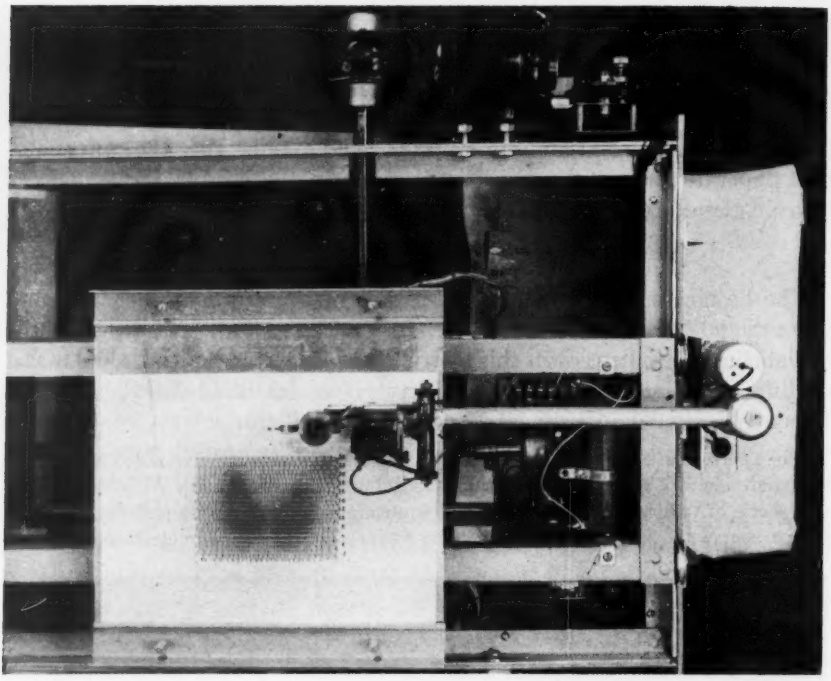

FIG. 2. Close-up view of the motor-driven writing platform showing, the fixed writing pen just above a completed gammagram (*see* text).

made, the sensitivity is found to be approximately thirty times that of the latter tube. The tube is attached to the under surface of the motor-driven scanning platform pictured in Figure 1. The circuits are so arranged that the ink-writing pen (Fig. 2) will deflect every 10, or 100, or 1,000 counts per second, depending upon the settings. The writing platform with the "directional" scintillation counter attached sweeps across the neck over the thyroid gland in the horizontal plane. The area to be scanned can be varied to suit the indication of the clinical problem. For example, when a substernal thyroid or a metastatic thyroid cancer is suspected, the entire neck and the upper thorax are included in the area scanned by the moving tube. On the other hand, if the thyroid gland alone is to be scanned, the area covered by the moving tube is restricted to a much smaller area, as shown in the completed gammagraph in Figure 2. The speed at which the platform and tube move may also be varied over a considerable range. Faster speeds are necessary in glands containing high activity, in order to prevent superimposing the deflections of the ink-writing pen, thus giving a clear definition of the variations in activity within the gland.

In order to obtain a gammagram of tissue containing a gamma-emitting radiotracer which permits maximum differentiation from the surrounding tissue and a clear definition of variations of activity within the parenchyma of the tissue, a combination of factors must be taken into consideration. The principal factor involved concerns the relationships of the rate with which the counter is moving over the thyroid gland, to the

rate of deflection of the writing pen over the most active part of the gland. Other contributing factors are the spacing between the horizontal parallel lines and the actual area scanned.

The counting rate is simultaneously recorded on a recording milliammeter. By comparing the similarly numbered lines of the gammagram (Fig. 2) with the corresponding numbered lines on the milliammeter tracing, a more quantitative interpretation of the variations in intensity of radiation within the parenchyma of the thyroid gland is possible. In Figure 3 are shown four curves recorded by the milliammeter, corresponding to the respective levels on the gammagram indicated by the solid connecting lines. Reading from above downward, as the "directional" scintillation counter moves from right to left, the curve rises abruptly when the edge of the right lobe is crossed and reaches a peak near the center of the lobe; it falls rapidly when this lobe is passed, only to rise again, but to a much lower level, as the small amount of radiating thyroid tissue in the upper left pole of the gland is crossed. The next curve shows two discrete peaks as the tube passes over the right and then the left lobe. The valley between the two peaks is deep, as in the first curve, since there is no radioiodine-containing tissue in the midline. In the third curve two discrete peaks are again seen, representing the activity of the right and left lobes respectively; however, the valley between the peaks is not as deep

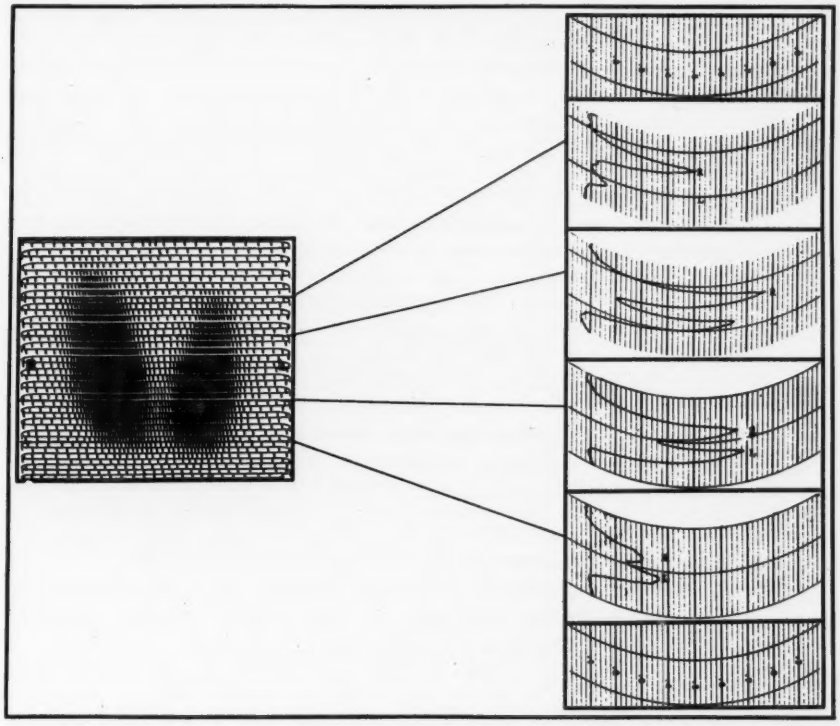

FIG. 3. Gammagram with four simultaneous milliameter records of the counting rate at the level of the gammagram indicated by the connecting lines (*see* text).

as in the preceding curves because the counter leaves the right lobe only to cross the thyroid isthmus, which contains radioactive iodine, though in a smaller quantity than the lateral lobes. As the "directional" counter begins to move over the left lobe, the second peak is written; and finally the curve falls to the base line as the lateral border of the gland is passed. In the final curve one sees two low peaks with a shallow valley, corresponding to the relatively less active inferior edge of the right and left lobes and isthmus.

The procedure for thyroid outlining followed in this laboratory is to determine first the time of maximum uptake as well as the maximum percentage uptake of radioactive iodine, using a 1-microcurie tracer dose. On the basis of this information as well as the clinical estimate of the approximate thyroid weight, the smallest amount of carrier-free radioactive iodine that will probably result in a concentration of 1 to 2 microcuries per gram of thyroid tissue is administered. The actual amount present per gram of thyroid tissue, however, is calculated from the percentage uptake of the outlining tracer dose. Experience has shown that an amount ranging from 1 to 2 microcuries per gram is necessary for a satisfactory gammagram. The patient returns for the gammagram at the time of maximum uptake and is placed in the proper position on an operating table, so that the skin of the anterior neck region is horizontal. The distal end of the "directional" scintillation counter is placed as close to the skin of the area to be scanned as possible. Usually the thyroid notch is the most anterior point. However, masses in the neck or increased anterior-posterior diameter of the chest may be the limiting factors. The neck and upper mediastinal areas are grossly scanned to determine the location of radioiodine-containing tissue. The scanning area is then reduced to include only the areas containing radioactivity, and these areas are re-scanned, usually at a slower speed. After completion of the gammagram and before the apparatus or the patient is moved, the aperture in the distal end of the scintillation counter is centered over the notch of the thyroid cartilage and the suprasternal notch. To facilitate accurate localization of these structures, they are marked with a skin-marking pencil. The points corresponding to these positions on the gammagraphic paper are noted in red ink. The scanning device is moved away from the patient, and the topographic anatomy is traced on a lucite drawing board placed horizontally on the neck, resting on the thyroid cartilage and chest. This sketch of the topographic anatomy of the head, neck and upper chest is then traced on cellophane paper. The points indicating the notch of the thyroid cartilage and the suprasternal notch, the primary reference points, are superimposed on their respective positions as previously recorded on the gammagram; and the anatomic localization of the areas of radioactivity is established. If lateral, oblique, or posterior gammagrams are indicated in order to localize more accurately the radioiodine-containing tissue or determine its size, the patient is put in the proper position and the process is repeated. Photostats of these completed gammagrams are filed, together with the interpretation, in the patient's chart. The time required by the entire procedure is approximately thirty minutes, depending upon the size and the activity present within the gland.

On all patients who came to surgery or necropsy, the gross and microscopic anatomy, as well as a radioautograph of the specimen, have been studied. The gross radioautographs, as well as the photographs of the surgical or necropsy specimen, are made from the cross-section of the unfixed frozen specimen. This is done in order to minimize shrinkage and distortion due to fixation and to make it possible to study radioautographically cystic nodules. The tissue is placed in apposition to x-ray film which is covered by a thin sheet of cellophane. The preparation is light-sealed and placed in a deep freeze for exposure.

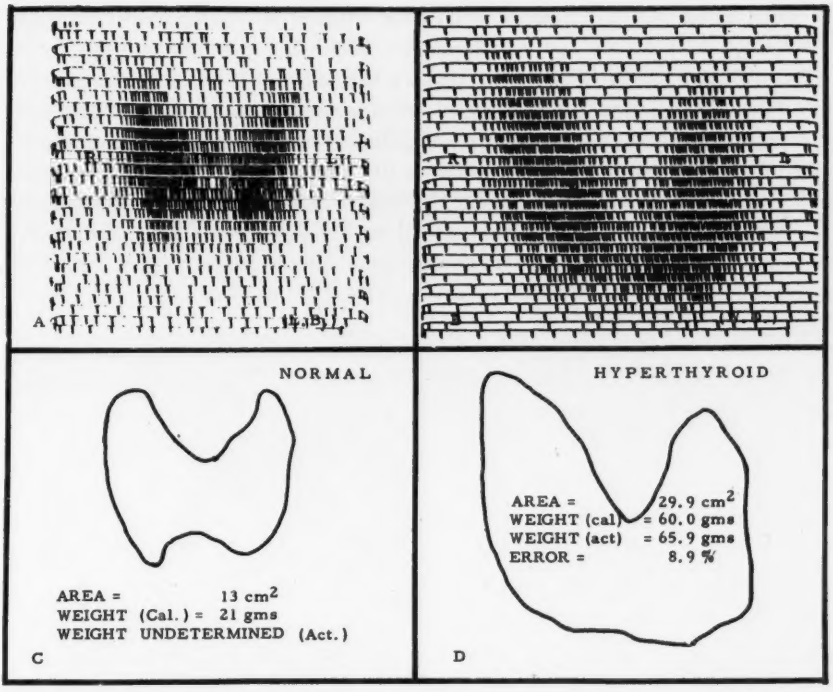

FIG. 4. Gammagrams of a normal (A) and of a diffusely enlarged thyroid gland (B). The perimeters of the gammagrams are traced in (C) and (D) (*see* text).

RESULTS

During the past fifteen months 175 gammagrams have been made on 68 patients. From this group a representative cross-section of the various clinical problems related to the differential diagnosis of masses in the neck have been chosen for presentation. Gammagraphic patterns demonstrating the relative size and shape of normal and abnormal thyroid glands are shown, together with photographs of the surgically removed or necropsy specimen, as well as the radioautograph of these specimens and the photomicrograph of the histologic section. For purposes of orientation Figure 4A shows a normal sized thyroid gland in a euthyroid patient (maximum thyroid uptake, 20.7 per cent of 1 microcurie of I^{131} administered) contrasted with the diffusely enlarged thyroid gland, (Fig. 4B) of a patient with hyperthyroidism (maximum thyroid uptake, 70 per cent of 1 microcurie of I^{131} administered). Figures 4C and 4D show the tracings of the perimeter of the normal and enlarged glands, respectively. These tracings are made by inspection and are necessary for the determination of the surface area

of the gland and the height of each lobe, both of which measurements are necessary to calculate the weight of the gland (9). Because of the subjective nature of this procedure for tracing the perimeter of the gammagram, the variation in calculated thyroid weight was determined in 62 gammagrams obtained from normal or from diffusely enlarged glands. The perimeter of each gammagram was drawn independently by three investigators. The weights calculated from the data obtained by these three investigators had a mean percentage variation of 6.5 per cent. To estimate the over-all errors of this method, gammagrams were made on two or more different occasions in 17 patients and the weights were calculated from the

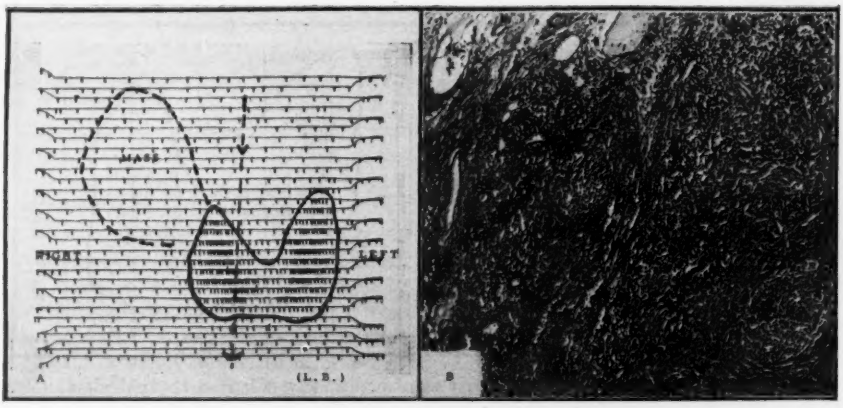

FIG. 5. Gammagram (A) of normal thyroid gland displaced by an extrathyroidal mass. (B)-photomicrograph from the surgical specimen.

surface area and height of the lobes. In these patients the mean percentage variation of the calculated weights of the thyroid gland at two or more different times in the same individual was 9.4 per cent, when the procedures were carried out by the same investigator. Thus we may conclude that the weight of the thyroid gland can be reproduced within 9.4 per cent, when calculated by the same individual on different occasions. From the foregoing data it can also be concluded that different individuals will agree within 6.5 per cent of one another in their calculation of the weight of the thyroid gland at any one time. The surface area and calculated weight of the normal and of the diffusely enlarged gland are shown in Figures 4C and 4D. The actual weight of the diffusely enlarged thyroid gland following total thyroidectomy was 8.9 per cent higher than the calculated weight. The normal thyroid gland was not removed.

Figure 5A shows a gammagraphic pattern produced by an extrathyroidal mass clinically indistinguishable from a nodule of the thyroid

gland. This gammagram shows a slightly deformed, normal sized thyroid gland displaced by the palpable mass to the left of the midline (indicated by the vertical dotted line which connects the notch of the thyroid cartilage superiorly with the suprasternal notch inferiorly). Figure 5B is a photomicrograph showing chronic inflammatory tissue found on biopsy of a

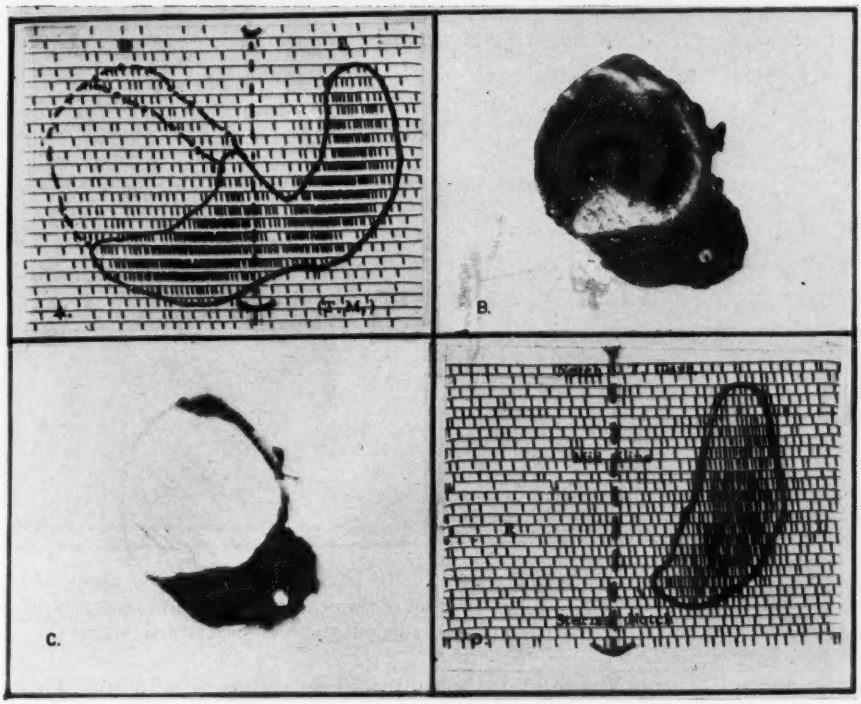

FIG. 6. Solitary nonfunctioning nodule in the right lobe of the thyroid gland. (A) Gammagram preoperatively, (B) cross-section of surgical specimen, (C) radioautograph of specimen shown in B, and (D) postoperative gammagram.

large lymph node which, at operation, was seen to be displacing the normal thyroid gland.

Gammagraphic patterns of benign, solitary, nonfunctioning nodules of the thyroid gland are presented in Figures 6 and 7. Figure 6A is the anterior gammagram demonstrating a large nonfunctioning nodule in the right lobe, compressing and displacing the remaining thyroid tissue to the left of the midline. Figure 6B is a photograph of the frontal cross section of the surgically excised right lobe. Figure 6C is a radioautograph of the specimen shown in Figure 6B. It is clear that the large mass is distorting

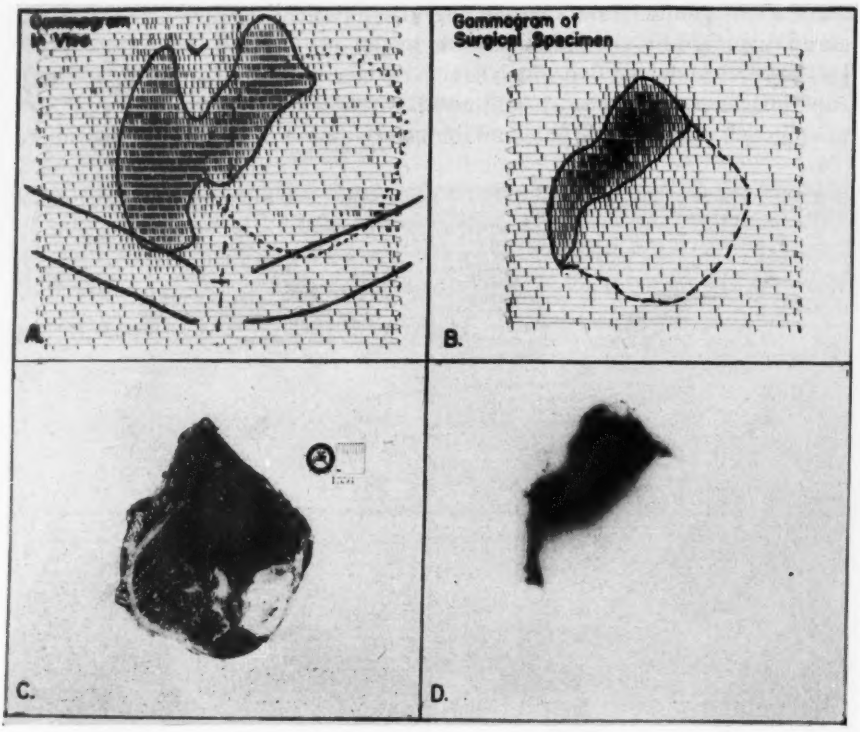

FIG. 7. Solitary nonfunctioning nodule in the left lobe of the thyroid gland. (A) Gammagram preoperatively, (B) gammagram of surgical specimen, (C) photograph of cross-section of surgical specimen, and (D) radioautograph of specimen shown in C.

the normal thyroid tissue and does not contain radioactive iodine. This was a simple cyst of the right lobe of the thyroid. Figure 6D is a gammagram made two days postoperatively (five days after the gammagram in Figure 6A); it demonstrates the remaining normal left lobe. This gammagram was made without further administration of radioactive iodine. Figure 7A is the anterior gammagram of a patient with a large palpable mass (outlined with the dotted line) deforming the left lobe, displacing the thyroid to the right, and extending inferiorly below the left clavicle. This was nonfunctioning tissue. Figure 7B is the gammagram of the intact surgical specimen to be contrasted with the photograph of the frontal cross-section of this specimen shown in Figure 7C and the radioautograph in Figure 7D. The clear differentiation between the normal radioiodine-concentrating gland and the nonfunctioning nodule is seen. Pathologically, this nodule consisted of a fetal adenoma. Figures 8 and 9 present different

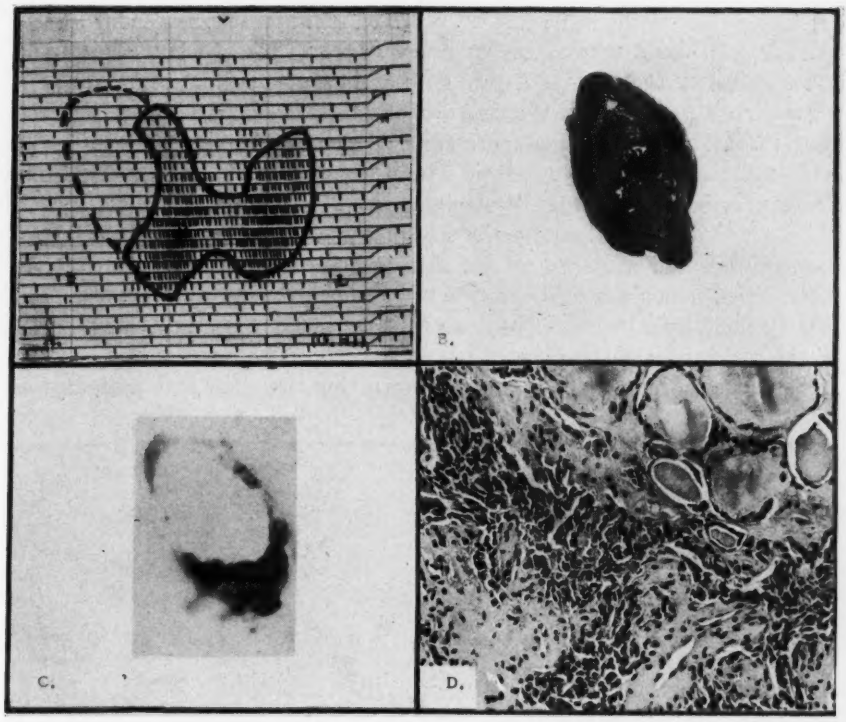

FIG. 8. Solitary malignant nodule in right lobe of the thyroid gland. (A) Anterior gammagram, (B) photograph of surgically removed right lobe, (C) radioautograph of cross-section of specimen in C, and (D) photomicrograph from the specimen shown in C, demonstrating the junction between normal and malignant tissue.

stages in the natural history of thyroid cancer. Figure 8A is the anterior gammagram of a patient with a solitary nonfunctioning malignant nodule in the right lobe of the thyroid. This gammagram is indistinguishable from those in Figures 6A and 7A. The surgically removed right lobe (Fig. 8B) demonstrates a multilobulated nodule surrounded medially and superiorly by normal thyroid tissue. Figure 8C is the radioautograph of the frontal cross-section of the specimen shown in Figure 8B, confirming the nonfunctioning character of the nodule surrounded by a narrow band of normal thyroid tissue. A photomicrograph (Fig. 8D) shows the junction between normal thyroid tissue and the papillary carcinoma.

Gammagrams of postoperative, recurrent and metastatic radioiodine-concentrating carcinoma of the thyroid are presented in Figure 9. Figure 9A is the anterior gammagram showing a small island of tissue in the bed

of the thyroid on the right side of the trachea. Figure 9B is a right lateral gammagram showing two discrete areas of radioiodine-concentrating tissue in the region of the angle of the mandible. Figure 9C is a posterior gammagram demonstrating that the larger of these two areas is deep in the neck near the midline, the other more superficial, probably located under the stump of the sternocleidomastoid muscle not removed at surgery three years previously. In order to exclude the possibility of lingual thyroid tissue, lead discs were cut to the size of the areas outlined in the lateral gammagram and attached to the skin in the corresponding locations. A lateral roentgenogram of the neck with the lead discs in position (Fig. 9D) clearly demonstrates that these areas are posterior to the pharynx and hyoid bone and are therefore not lingual tissue. The maximum uptake of radioactive iodine by the iodine-concentrating tissue in this patient was

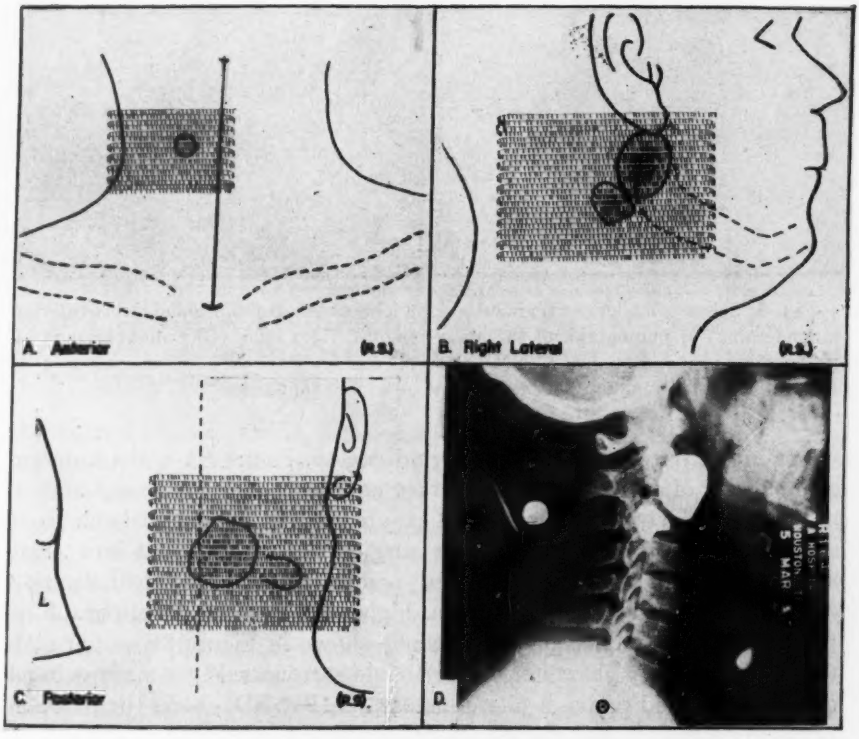

FIG. 9. Recurrent and metastatic carcinoma of the thyroid. (A) Anterior gammagram, (B) right lateral gammagram, (C) posterior gammagram, and (D) right lateral roentgenogram of the neck with lead discs attached to the skin, corresponding to the size and location of the radioiodine-concentrating tissue outlined in A, B and C (*see* text).

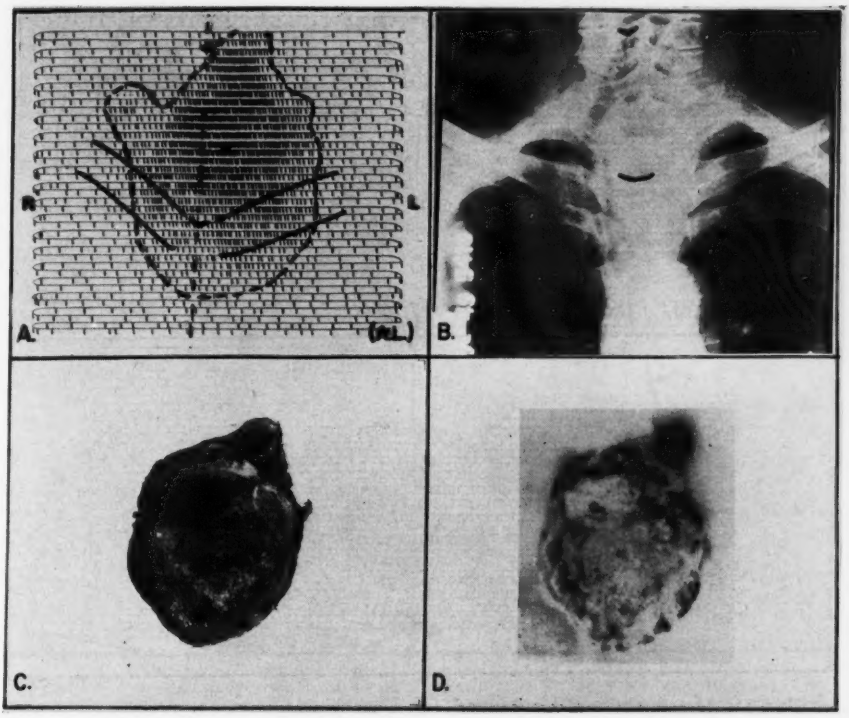

FIG. 10. Multinodular substernal thyroid, (A) Anterior gammagram, (B) roentgenogram of upper chest demonstrating a superior mediastinal mass, (C) photograph of cross-section of the surgical specimen, and (D) radioautograph of the specimen in C (*see* text).

only 0.6 per cent, increasing to 1.1 per cent following administration of thyrotropic hormone. Because of the very low uptake of radioactive iodine, the dose required for these gammagrams was 1,000 microcuries. The weight of the radioiodine-concentrating tissue was estimated by making plastic models to conform to the size of the areas shown in the gammagrams taken anteriorly, laterally and posteriorly. From the volume of the plastic models the total weight of tissue was calculated to be approximately 7 grams.

Figure 10 and Figure 11 represent gammagraphic patterns of multinodular thyroid glands. The appearance of these gammagraphic patterns should be contrasted with those of the uninodular gland shown in Figures 6, 7 and 8. The complete loss of thyroid configuration in these multinodular glands is felt to be characteristic. Figure 10A is the anterior gammagram of a multinodular substernal thyroid gland. The usefulness of the gammagram in this case is apparent when one considers that this patient

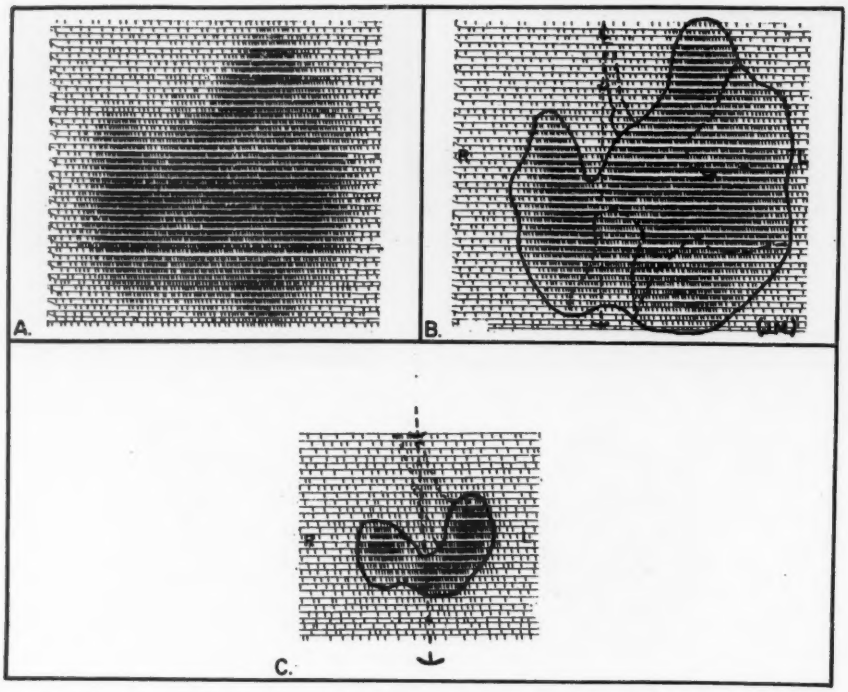

FIG. 11. Multinodular thyroid gland. (A) Untouched gammagram, (B) gammagram with perimeter drawn and hypofunctioning nodules as well as pyramidal lobe indicated by dotted lines, and (C) postoperative gammagram.

had myasthenia gravis, and the physician had to decide whether the superior mediastinal mass compressing and displacing the trachea (Fig. 10B) was a thymic tumor or a substernal goiter. The photograph of the surgical specimen and the radioautograph of this specimen are shown in Figures 10C and 10D, respectively; they agree well with the size and appearance of the grammagram.

Gammagrams of a large multinodular gland are shown in Figure 11. Figure 11A is the untouched anterior gammagram demonstrating the distortion of the normal thyroid configuration. In Figure 11B the perimeter of the gland, including a small pyramidal lobe, is traced and the areas representing hypofunctioning nodules enclosed in dotted lines. This interpretation of the gammagram was confirmed by radioautography of the surgical specimen. Figure 11C is a postoperative gammagram demonstrating residual thyroid tissue, including the pyramidal lobe. It is of physiologic interest that the maximum thyroid uptake of the 1 microcurie

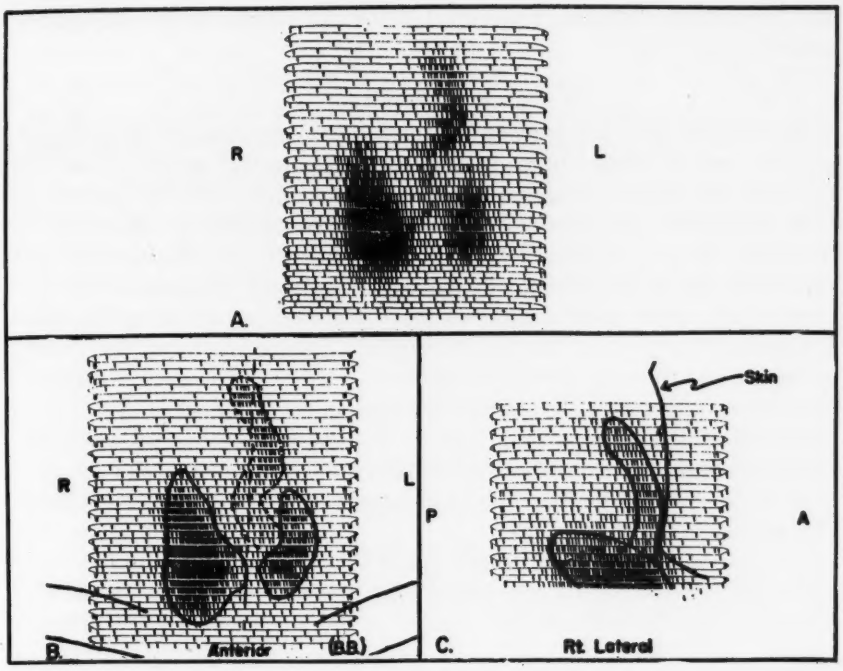

FIG. 12. Postoperative recurrent hyperthyroidism. (A) Anterior gammagram, (B) anterior gammagram with perimeters drawn—note the pyramidal lobe—and (C) right lateral gammagram (*see* text).

of radioactive iodine administered before and after operation on four different occasions over a period of seven months, ranged between 15 and 20.1 per cent in spite of the large mass of tissue removed.

The usefulness of the gammagram in determining the extent of prior surgery of the thyroid gland is demonstrated in Figure 12. This patient had a subtotal thyroidectomy for hyperthyroidism due to diffuse toxic goiter ten years before this study. Symptoms of hyperthyroidism returned several months prior to these observations, at which time the maximum uptake of 1 microcurie of radioactive iodine was 60.8 per cent. Figure 12A is the untouched anterior gammagram showing the residual functioning thyroid tissue in the right and left lobes and in a large pyramidal lobe. The lateral gammagram shown in Figure 12C was made so that the depth of this tissue could be determined. It is obvious that the weight of such a thyroid gland could not be calculated; therefore, a plastic model of the residual thyroid tissue was made, using the anterior and lateral gammagrams as guides in a manner similar to the procedure discussed previously.

The weight thus calculated was used in the calculation of the therapeutic dose of radioiodine.

DISCUSSION

The automatically recording motor-driven "directional" scintillation counter used in these studies facilitates a quantitative evaluation of the size, the anatomic configuration and relationships, and the functional state of different portions of the diseased thyroid gland. In other words, the gammagraphic pattern is a composite picture of what the surgeon and anatomist would see after dissection, combined with the information the physiologist could provide after study of the radioautographs. The close quantitative agreement between the *in vivo* gammagram and the photograph of the surgical or necrospy specimen, as well as the radioautograph, has been demonstrated. In the past four years several reports (10–13) have described the qualitative approach to this problem, using manually operated instruments of much lower sensitivity. The importance of accurate quantitative values for the weight of thyroid tissue in the calculation of therapeutic doses is apparent.

On the basis of available data it is felt that the gammagraph can be of aid to the clinician in the differential diagnosis of masses in the neck. The following clinical conditions are thought to produce characteristic gammagraphic patterns: 1) solitary intrathyroidal nodules, hypofunctioning or hyperfunctioning; 2) multiple intrathyroidal nodules; 3) extrathyroidal masses displacing the normal thyroid gland; and 4) substernal thyroid extension. Furthermore, the gammagraph is useful in the localization and estimation of the weight of aberrantly placed or metastatic thyroid tissue which concentrates radioiodine. Finally, the extent of previous thyroid surgery can be determined.

The frequency with which the pyramidal lobe can be demonstrated is of interest. Two patients, who have recently been studied because of the problem of recurrent hyperthyroidism, had enlarged pyramidal lobes and called attention to this problem. The role of this lobe in the genesis of recurrent postoperative hyperthyroidism is not understood. That it frequently remains after thyroidectomy has been noted several times.

One point in the procedure deserves emphasis as a possible source of error—that is, the distance from the aperture of the "directional" scintillation counter to the skin of the neck region. This distance must be short and constant if accurate and reproducible gammagrams are to be obtained. From *in vivo* observations, the distortion of the gammagram, and therefore of the calculated weight, resulting from positioning the aperture of the tube at increasing distances from the skin of the neck, was determined.

When the aperture was 0.5 cm. from the skin, the calculated weight was 38.6 Gm.; at 5.0 cm., 66.3 Gm.; and at 10.0 cm., 78.9 Gm., representing increases of 72 per cent and 104.6 per cent, respectively, over the weight at 0.5 cm. Errors of this magnitude are of great significance when therapeutic doses of radioiodine are calculated on the basis of thyroid weight.

SUMMARY

1. Gammagraphic patterns of the thyroid gland as an aid in the differential diagnosis of masses in the neck are presented.

2. Photographs of surgical or necropsy specimens, together with radioautographs of these tissues, demonstrate the accuracy of the gammagraph in portraying the size, configuration and functional state of the nodular thyroid gland.

3. The instrument used, as well as procedure followed in this laboratory, are described in detail.

4. The accuracy and reproducibility of the technic, as well as the possible sources of error, are discussed.

Acknowledgment

The authors wish to express their appreciation to Mr. Royal H. Benson, B.S., for his assistance in these studies and particularly for the many improvements in technic which he developed and which have been incorporated in the procedure.

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THE LABORATORY DIAGNOSIS OF EXTRA-THYROIDAL HYPERMETABOLISM*

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ELEVATION of the basal metabolic rate is frequently misleading in the evaluation of suspected hyperthyroidism. This may be because the BMR indicates total body metabolism and represents the end result of a great many oxidative processes, only one of which is due to the effect of thyroid hormone. Cerebral stimulation, increased metabolism of malignant cells, pregnancy, pheochromocytoma, alterations in hemodynamics such as occur in congestive heart failure, and many other nonthyroid states may notably affect the metabolic rate. Thus, the *result* is less in error than is its *interpretation.*

This discussion is concerned with the laboratory evaluation of patients referred to a thyroid clinic because of an elevated BMR and in whom the diagnosis of hyperthyroidism was usually considered, if not actually made, by the referring physician. These patients were hospitalized for several days while thyroid metabolic studies were carried out. Many patients were eliminated from this series when previously elevated basal metabolic rates fell to normal levels as a result of hospitalization.

With certain exceptions these patients were difficult diagnostic problems. Many had symptoms suggestive of toxicity, such as nervousness, weight loss, increased sweating, palpitation and easy fatigability. Some had diffuse enlargements of the thyroid gland, some had nodular growths, whereas in others the thyroid was not enlarged. In all cases hypermetabolism was present, as reflected by an elevated BMR, but was shown to be due to *extrathyroidal* disease rather than hyperthyroidism. The efficacy of additional laboratory tests in establishing the diagnosis of extrathyroidal hypermetabolism represents the basis of the following discussion.

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PROCEDURE

The laboratory tests used to study these patients from a metabolic standpoint were the BMR, SMR (somnolent metabolic rate (1)), serum PBI (protein-bound iodine), RAI (radioactive iodine) and serum cholesterol levels. All BMR determinations were made with the patient in bed under conditions which were as basal as possible. Usually an oral barbiturate was given the night before but never on the morning of the test. Immediately after completion of the BMR blood was drawn for the PBI and cholesterol assays; then a somnolent metabolic rate determined. After the patient awakened, a tracer dose of 5 to 15 microcureis of I^{131} was given. At the end of twenty-four hours the patient was taken to the radioiodine laboratory, where the percentage of the administered I^{131} found in the thyroid gland and in the urine was determined.

The PBI (2) and SMR (1) values were obtained by methods previously described. The early RAI determinations were made with a bismuth plate tube (3), but later a scintillation counter was employed; distances from gland to tube were 15 and 20 cm., respectively.

RESULTS

In central Ohio, the normal BMR is -5 per cent with a standard deviation of ± 8 per cent (4). Values of $+4$ per cent or above were included in this study. No normal values have been established for the SMR; we have

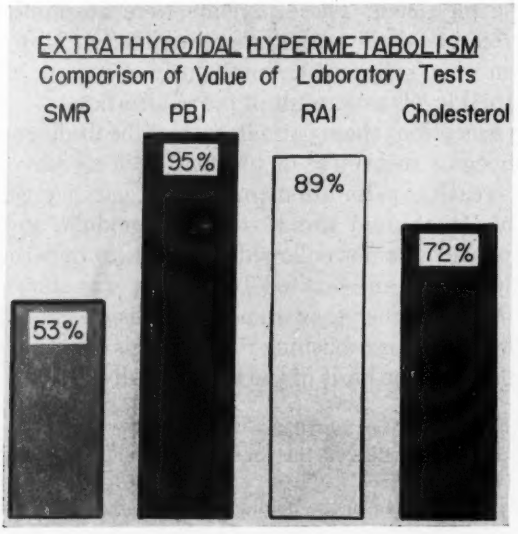

FIG. 1. Relative effectiveness of laboratory tests in ruling out hyperthyroidism.

TABLE 1. AVERAGE VALUES OBTAINED WITH FIVE LABORATORY TESTS, IN THE SIX MOST COMMON CONDITIONS CAUSING EXTRATHYROIDAL HYPERMETABOLISM. (THE SMALLER FIGURES DENOTE THE NUMBER OF PATIENTS.)

	BMR	SMR	PBI	RAI	Cholesterol
Leukemia	+57	+36	5.1	27	142
6 patients	6	6	5	6	4
Diabetes	+52	−7	4.7	23	199
3 patients	3	3	2	2	3
Miscellaneous	+19	−4	4.9	37	170
18 patients	18	18	13	12	16
Airway obstruction	+17	0	4.3	19	201
5 patients	5	5	5	5	4
Cardiovascular disease	+17	−3	3.9	27	211
5 patients	5	5	4	3	5
Psychoneurosis	+16	−15	4.1	28	171
11 patients	11	11	9	8	9

considered our upper limit of normal to be −7 per cent, and the statistics which follow are based on this figure. The upper normal value of the PBI has been previously set at 6.7 micrograms per cent (1). Uptake of radioiodine by the thyroid gland below 40 per cent (5) and serum cholesterol levels above 150 mg. per cent were considered outside the range of values found in hyperthyroidism. It should be stressed that although the upper limit of normal for each of these determinations represents only a point on a line, some figures must be used to establish the upper limits in order to proceed with the study.

Figure 1 illustrates the relative effectiveness of the additional laboratory tests as an aid in the diagnosis of extrathyroidal hypermetabolism. In order, the values were: PBI, 95 per cent; RAI, 89 per cent; cholesterol, 72 per cent; and SMR, 53 per cent. It should be pointed out that the BMR was 0 per cent (or 100 per cent nondiagnostic).

The average values of the five laboratory tests in our six most common causes of extrathyroidal hypermetabolism are tabulated in Table 1. Note that most of the values are within normal limits, excepting, of course, those for the BMR.

DISCUSSION

It has long been the aim of the thyroidologist to have available one single test which would exactly evaluate the functional state of the thyroid

gland. However, thyroid physiology is so intricate that no one test has yet been devised which approaches this ideal. It is of fundamental importance to understand that the various tests cannot be compared among themselves, since each measures a *different aspect* of thyroid metabolism. Therefore, we must be cognizant of the specific function of the thyroid gland that is measured by each laboratory procedure. This is shown diagrammatically in Figure 2.

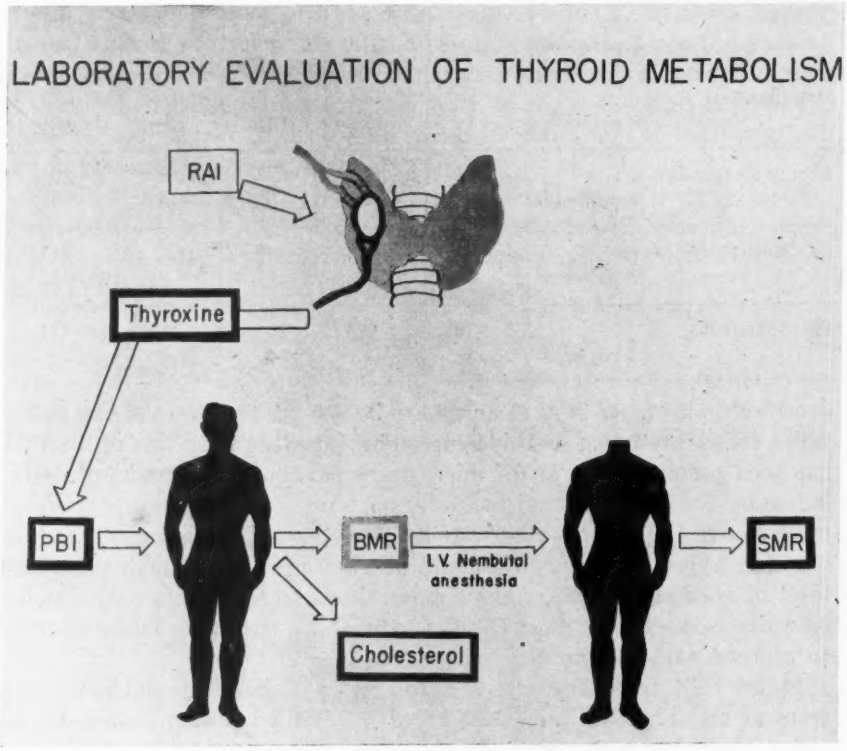

FIG. 2. Diagrammatic representation of factors measured by various tests of thyroid function.

The RAI uptake, determined by a single 24-hour count, tells only how much radioactive iodine is in the thyroid gland at that time. It indicates neither the rate of uptake nor the rate of release. Serial counts are required for this additional information. The RAI is not elevated by the common functional thyroidal disorders nor by organic nonthyroid diseases. However, the effect of exogenous iodination in obscuring the results of tracer studies is well known, and the source of iodination is often diffi-

cult to discover. Moreover, as yet, the use of radioiodine is still restricted to certain specialized centers.

The serum PBI measures the amount of circulating iodine bound to the protein molecule. The major portion is generally accepted to be calorigenic substance and thus this test theoretically gives the most direct measure of the formation of thyroxine. It is not affected by the majority of diseases producing extrathyroidal hypermetabolism (6). However, it is not a gener-

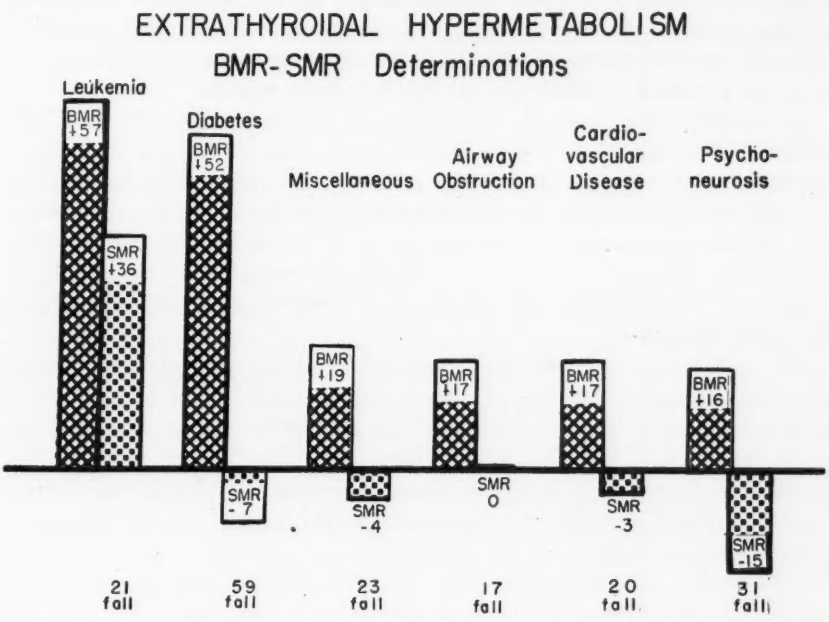

FIG. 3. Comparative determinations of the basal and somnolent metabolic rates.

ally available procedure and, like RAI uptake, is notably affected by exogenous iodination.

The BMR has been generally accepted to be the test of choice if only one metabolic test is available. However, a BMR measures total body metabolism and no matter how basal the test may be, cannot differentiate between possible etiologic factors in the hypermetabolism. Furthermore, serial BMR's must often be determined before a patient is sufficiently acclimated to assure a reliable and dependable tracing.

The SMR is a metabolic rate performed under an ideal basal state. It is especially valuable in functional disorders, such as anxiety neuroses, since it is "void of all nervous and muscular factors" (7). Moreover, in many patients with hyperthyroidism the disease is often complicated by

varying amounts of underlying nervousness, which adds to an already elevated BMR.

The seemingly low value of 53 per cent compatibility with the diagnosis, in the case of the SMR (Fig. 1) requires an explanation. In diagnostic clinics the confusing cases of extrathyroidal hypermetabolism are not those of leukemia or diabetes, but rather the psychoneuroses. In our group of 11 such patients, the SMR was −6 per cent or above on only three occasions, making a compatibility of 72 per cent. *The highest SMR was −1 per cent.* Moreover, except for 3 diabetic patients, the average fall of the SMR was the greatest in psychoneurosis, being 31 per cent (Fig. 3). It is in this group of patients that the SMR is most useful.

In addition to its value in establishing a primary diagnosis, an SMR determination serves as a base line for comparing the results of various kinds of therapy. The SMR will give a truer, more reproducible value than a BMR, which frequently fluctuates from day to day (1). The administration of iodine, of thiouracil derivatives, or of therapeutic doses of radioiodine does not interfere with the SMR determination. Thus we have found the SMR to be the best single test for following all kinds of therapy for hyperthyroidism.

The concentration of serum cholesterol has been used for many years to reflect inversely the rate of thyroid activity. At best it is an indirect test and is usually more useful when used serially in evaluating the results of therapy, than in establishing a diagnosis. The daily variance of the cholesterol determination further discourages our use of this test. It has been our experience that the serum cholesterol level is usually normal in hypothyroidism and in hyperthyroidism, as well as in euthyroidism. Consequently, the high percentage of compatibility with the diagnosis obtained in our group of euthyroid patients is misleading.

Finally, it should be re-emphasized that, despite the increasing use of laboratory methods to determine metabolic states, the reliability of these methods has been overemphasized. Clinical judgment remains the best single method of establishing a final diagnosis of thyroid function. The laboratory becomes of increased value in the study of borderline states or in confusing clinical pictures, such as have been considered in this discussion.

SUMMARY

For purposes of differential diagnosis, determinations of the basal metabolic rate, somnolent metabolic rate, uptake and excretion of radioiodine, and concentrations of serum protein-bound iodine and of cholesterol have been made in a series of 49 patients with hypermetabolism. All patients had an elevated BMR and many entered the clinic with an initial

diagnosis of hyperthyroidism. With the aid of the foregoing tests, all patients were proven to have *extrathyroid hypermetabolism.* An evaluation of these procedures has been made in order to determine their effectiveness in establishing this diagnosis. It has been emphasized that these tests cannot and *should not* be compared among themselves, since each test measures a different aspect of the complex metabolism of the thyroid gland.

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GOITER PREVENTION WITH IODIZED SALT: RESULTS OF A THIRTY-YEAR STUDY*

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THE State of Michigan, situated as it is in the Great Lakes endemic goiter belt, has offered an opportunity for the study of the disease and the effects on its incidence of iodized salt used as a prophylactic measure. This challenge was accepted by the Michigan State Medical Society and the State Department of Health thirty years ago, and the story of their accomplishments in goiter prophylaxis since that time is comparable to the classic discoveries of preventive medicine in many other fields. A review of their experience clearly shows that endemic goiter is truly one of the most easily preventable of known diseases.

Iodine was used incognito in the form of sea weed and sea sponge by the ancient Chinese and Greeks. Roger of Palermo (1) in the year 480 observed that sea sponges were of benefit for goiter, and Paracelsus in the 16th century believed that goiter was due to a deficiency of minerals in the drinking water.

In 1812 Courtois of France isolated the element iodine and described its unusual color and vapors. It was soon recommended for the treatment of goiter and Switzerland used iodized salt as a prophylactic measure as far back as 1840. Chatin (2) in 1850 showed the iodine content of the soil and water to be low in goiter areas and noted also that goiter was improved by sea sponge ash. In 1896 Baumann (3) discovered iodine to be present largely in the thyroid gland.

Beginning in 1907, the work of Marine (4) and his associates in the study of goiter and its relation to iodine marks the advent of the newer and more practical knowledge of this relationship. They showed that when brook trout were maintained without iodine, goiter invariably developed and that the addition of a small amount of iodine to the water caused the goiters to decrease in size. This was shown to be true also in the case of cattle, sheep,

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This article will be included in the bound volume of the 1952 "Transactions of the American Goiter Association," published by Charles C Thomas, Publisher, which will be available for sale early in 1953.

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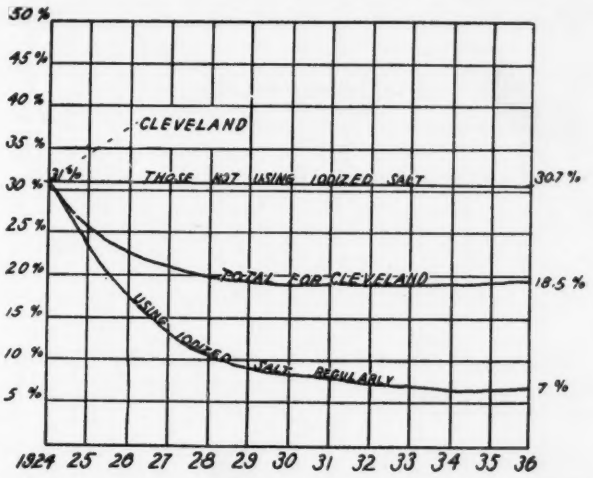
pigs and other animals. The result of a deficiency of iodine was soon recognized in the field of animal husbandry; Hart (5) described the malady of "hairlessness in newborn pigs," and Kalkus (6), the "weakness" and "big neck" in newborn lambs and colts. Iodine was soon added to the rock salt in these areas and later other minerals such as manganese, iron, cobalt and copper. It is difficult to believe, but it is true, that the animals have received better therapy than the people in many goiter areas.

In 1916 Kimball (7) studied the incidence of goiter in the school children of Akron. This study of 10,000 children continued for four years and showed that in only 0.2 per cent of those who added a small amount of



Incidence of goiter in Cleveland from 1924 to 1936.

FIG. 1. (Reprinted, by courtesy of publishers and author, from Kimball, O. P.: Goiter prevention in Michigan and Ohio, *J.A.M.A.* **108:** 860–864 (March 13) 1937.)

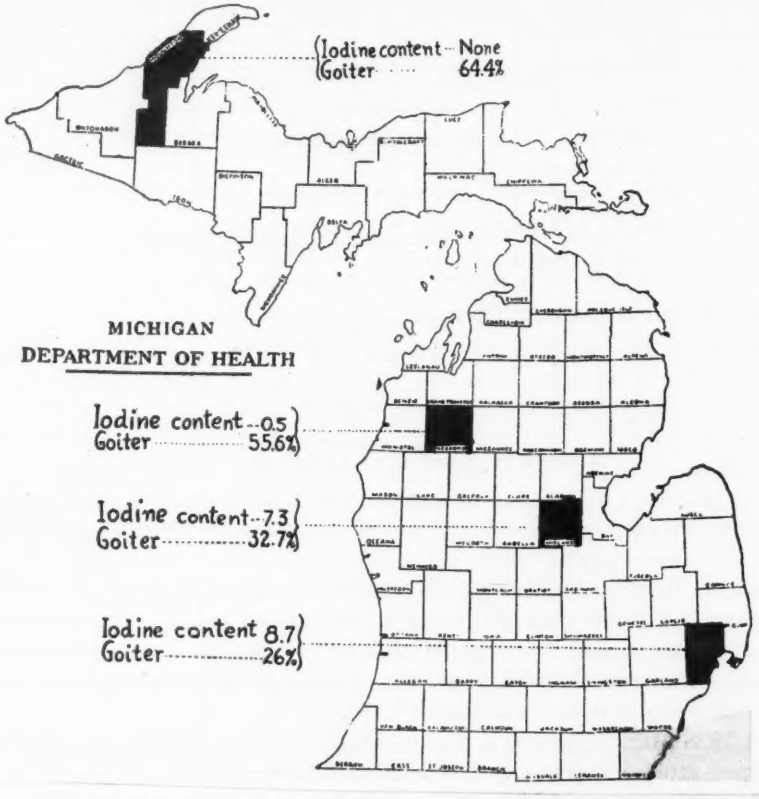
iodine to their diet did any enlargement of the thyroid gland develop, whereas in 27.6 per cent of those who did not add iodine, there was recognizable overgrowth of the gland. In the group that showed increase in size on first inspection, 79.7 per cent of the enlargements decreased when the deficiency of iodine was made up (Fig. 1).

In 1928 Marine (8) , as a result of his studies, believed that goiter is a compensatory hypertrophy of the thyroid resulting from a relative or an absolute deficiency of iodine. This deficiency of iodine may be due to: 1) factors which bring about an abnormally low intake of iodine, 2) factors which interfere with absorption or utilization of an otherwise adequate intake, or 3) factors which increase the needs of the body for the iodine-

containing hormone. The chief factor in Group 1 is the lack of iodine in the food and water supply. Factors in Group 2 are becoming better known, as the complex interrelationships of the glands of internal secretion are becoming unraveled. Concerning Group 3, it is well known that the thyroid is prone to enlarge with puberty, menstruation, pregnancy, and with certain infections; but these may represent merely a need for more thyroxine when an inadequate supply of iodine for its manufacture is available.

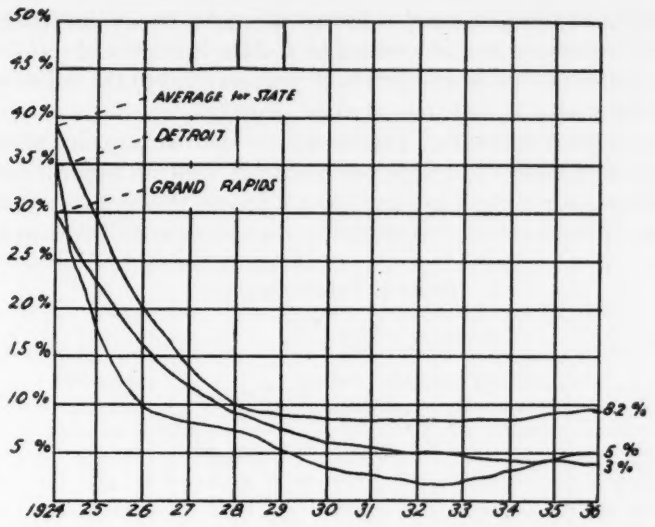
EXPERIENCE IN MICHIGAN

Serious consideration of the goiter problem in Michigan was the result of the findings by draft boards during World War I: "Goiter was so prev-



Goiter survey of school population: Iodine in water estimated in parts per billion.

FIG. 2. (Reprinted, by courtesy of publishers and author, from Kimball, O. P.: Goiter prevention in Michigan and Ohio, *J.A.M.A.* **108**: 860–864 (March 13) 1937.)



Incidence of goiter in Michigan from 1924 to 1936.

FIG. 3. (Reprinted, by courtesy of publishers and author, from Kimball, O. P.: Goiter prevention in Michigan and Ohio, *J.A.M.A.* **108**: 860–864 (March 13) 1937.)

alent that in some groups as high as 30 per cent of the persons were incapacitated for Army Service, owing to disqualifying goiters in 583 registrants" (9).

The studies in Michigan began in 1923 and have been a cooperative effort of the State Medical Society and the Department of Health with the guidance of Dr. O. P. Kimball and the cooperation of the salt manufacturers and wholesale grocers. The Medical Society has had an Iodized Salt Committee for the purpose of continuous study of the problem and the early work of Drs. D. M. Cowie, Roy D. McClure, O. P. Kimball, Slemons, Olin (10) and Towsley is noteworthy. Early in 1924, after the first survey was completed, a program of goiter prevention was outlined and a campaign by the State Department of Health was conducted to emphasize the fundamental causes of endemic goiter.

Four counties were selected because of their position across the state and their agricultural and soil differences. At the first survey in 1923 all of the school children up to the eighth grade were examined for thyroid enlargement by personnel of the Health Department, and water and soil samples from various areas were tested for iodine content. At surveys in 1928, 1935, and 1951, information was also obtained as to whether the children had been using iodized salt regularly, irregularly, or not at all.

The results of the first survey showed beyond a doubt that there was a correlation between a lack of food iodine and the incidence of simple goiter, since the goiter incidence was inversely proportional to the iodine content of the water supply in the areas studied (Fig. 2).

The subsequent surveys in 1928 and 1935 showed a remarkable reduction in goiter among those using iodized salt with an over-all reduction of the incidence from 38.6 per cent to 8.2 per cent (Fig. 3). In those who had used the iodized salt the incidence was reduced to between 2 and 3

TABLE 1. THYROID SURVEY

May—1951

County	Total examined	Normal thyroid	Per cent	Abnormal thyroid	Per cent
Macomb	34,293	34,111	99.5	182	0.5
Houghton	6,930	6,355	91.7	575	8.3
Wexford	3,811	3,798	99.7	13	0.3
Midland	8,751	8,734	99.8	17	0.2
Total	53,785	52,998	98.6	787	1.4

per cent, whereas in the group who had not used it the incidence remained at from 25 to 35 per cent. A valuable study was possible in the mining town of Calumet, where in 1932–1935 during the depression, two thirds of the families were on relief and used noniodized bag salt. The increase in the number of goiters following this use of noniodized salt was most striking—up to 43 per cent.

The 1951 survey showed an incidence of goiter of 1.4 per cent in 53,785 students compared with an incidence of 38.6 per cent in 65,537 students examined in 1924 (Table 1). The results in a typical county, Wexford, are shown in Figure 4.

DISCUSSION

The fact should be emphasized that at no time has there been a law compelling people to use iodized salt. The State Department of Health and the State Medical Society have carried on a program of education and advice in the form of news articles, placards, and health talks, but we have found that unless this problem is kept continually before the people there is a great tendency for the sale of iodized salt to decrease. Mrs. Alice H. Smith of the Nutrition Section of the Health Department recently surveyed the grocery stores in these counties and found that the iodized salt was not displayed as prominently as we should like, and in some instances

had to be specially ordered. This problem has been corrected in Switzerland and Canada by having all table salt enriched with iodine. The report of McClendon (1) has shown the remarkable results of goiter prophylaxis with iodized salt in Switzerland.

Curtis and Fertman (12) have performed iodine balance studies and as a result of these the Food and Nutrition Board of the National Research Council gives the daily requirement of iodine for an adult as 0.15 to 0.30 milligrams. Iodized salt meets this requirement and is especially important in adolescence and during pregnancy.

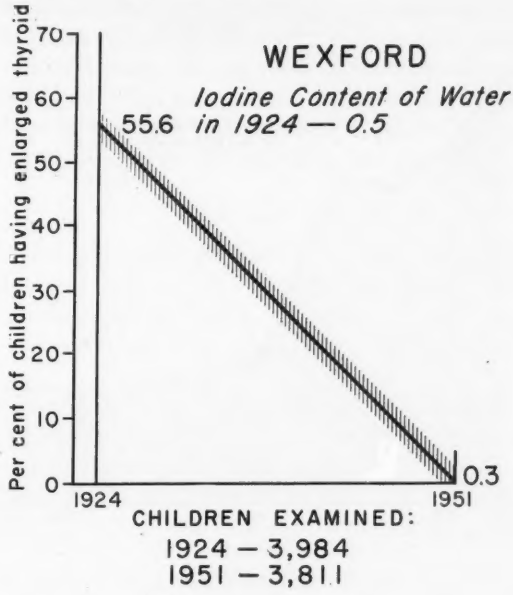


FIGURE 4

No ill effects have been noted from the use of iodized salt. The small amount of iodine used has not, according to the American Dermatological Society, caused any difficulty in those with a tendency to acne, an objection which was once raised. The stabilizers used insure a uniform mixing of the iodine and the Canners' Association has stated that there is no disadvantage in using iodized salt in all types of food canning. Moore (13) of the Division of Syphilis at Johns Hopkins Hospital reported that 10,000 patients were treated with large doses of iodides but in not a single instance did iodine hyperthyroidism develop.

It is well known that there are present in some areas certain goitrogenic agents, such as are present in cabbage and other vegetables. Chesney and

Webster (14) and Helwig (15) have shown that when additional iodine is given to rabbits receiving an otherwise goitrogenic diet, goiter does not develop.

Arnold Jackson (16) in 1926 stated that "simple adenoma is one form of endemic goiter which closely follows colloid goiter in regional distribution. The incidence of adenoma of the thyroid is decreased by the prophylactic treatment of colloid goiter."

McClendon (17), using draft board statistics, has shown that the incidence of exophthalmic goiters throughout the United States is proportional

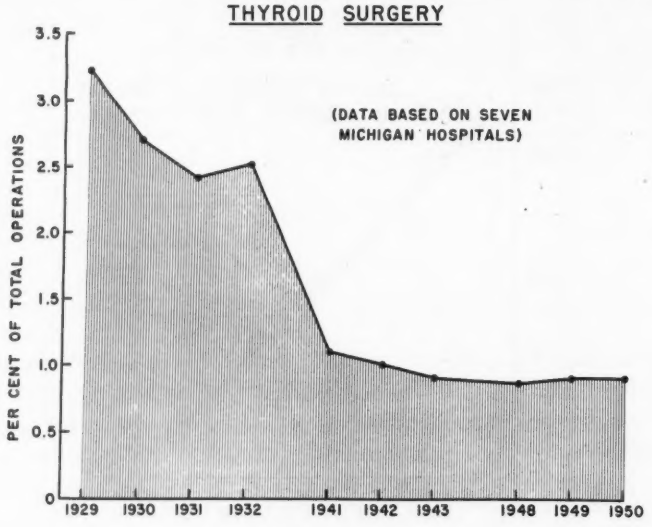


FIGURE 5

in every locality to the incidence of endemic goiter. McClure (18) reported that since the introduction of iodized salt, coincident with the great dropping off in the number of enlarged thyroids there has been a dropping off in the number of operations for hyperplasia and adenomata of the thyroid in seven large Michigan hospitals (Fig. 5).

Among the interesting findings of the study was the fact that there was a tremendous difference in the incidence of goiter in areas only a few miles apart. At Mount Clemens in 1924, 26 per cent of the children had enlarged thyroids, while at Romeo only twelve miles distant, 75 per cent of the children had goiter. The water supply at Mount Clemens contained 25 parts of iodine per billion, whereas at Romeo there was only a trace of iodine in 50 liters.

The wide use of an enriched rock salt by stock farmers has undoubtedly

been a factor in some of these essential elements finding their way into the food, thus helping to reduce the incidence of endemic goiter.

W. H. Sebrell (19), Director of the National Institutes of Health, has cited surveys made by the Public Health Service which show that goiter is quite prevalent in areas outside the so-called "goiter belt"—"In Mitchell County, Georgia, 31 per cent of women had an enlarged thyroid gland and in Alachua County, Florida, 25.6 per cent of the negro females between 13–20 years of age were affected."

Natural salt deposits contain varying amounts of iodine. An incidence of conversion from a nongoiter area to a goiter area occurred in the Kanawha River Valley of West Virginia. Kimball (20) reported that prior to 1900 a local crude, coarse, brown salt was used and goiter was exceedingly rare. After this, a sparkling white salt containing no iodine was shipped in and preferred. The goiter rate rose sharply and in 1922 a survey showed that 60 per cent of adolescent girls in the valley had goiter.

CONCLUSIONS

1. Salt can be iodized, accurately, efficiently and inexpensively.
2. The incidence of endemic goiter has been reduced almost to nil since iodized salt has been so widely used.
3. We have seen no subject who showed the slightest ill effects from the use of iodized salt.
4. Our experience leads us to believe that toxic nodular goiter and diffuse goiter are less apt to occur when there has been no previous enlargement of the gland, such as occurs in endemic goiter.
5. It is essential that departments of health and medical societies continually remind the public of the importance of this prophylactic measure.

Acknowledgment

Acknowledgement is gratefully made to the Michigan Department of Health, who conducted these surveys.

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RECURRENT HYPERTHYROIDISM AFTER ANTITHYROID THERAPY AND THYROIDECTOMY*

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SINCE June 1943, all patients with hyperthyroidism treated at the Lahey Clinic have been prepared for operation with the use of antithyroid drugs, and radical subtotal thyroidectomy has been performed. This method of treatment has been applied both to primary hyperthyroidism and to nodular goiter with hyperthyroidism, with the only difference that Lugol's solution has been employed during the latter part of the preparation in patients with primary hyperthyroidism. During this time approximately 2,450 patients have been so prepared and operated on; of this number, approximately 80 per cent had primary hyperthyroidism and 20 per cent nodular goiter with hyperthyroidism. Sufficient time has elapsed since this method of management was adopted to evaluate the results of treatment. In a consideration of the results of this treatment the problem of recurrent hyperthyroidism has been studied, and this report will be concerned with the incidence and treatment of recurrent hyperthyroidism in the first 1,800 patients treated before July 1949. This study was undertaken to determine whether there had been any change in the incidence of this complication since the advent of the use of antithyroid agents, in comparison with the previous method of management.

We have reviewed the pertinent literature since our previous report (1) in 1939 for the ten-year period up to 1949 (Table 1). The rate of recurrence reported varied from 2 to 12 per cent. In the original study of Cattell and Morgan (1) from the Lahey Clinic in 1939, 4,956 patients were reported, with an incidence of recurrent and persistent hyperthyroidism of 3.3 per cent. In the next largest series studied by Troell (2), 1,397 cases were reported, with an incidence of 6 per cent. In a group reported by Hurxthal (3) in 1945, covering 643 patients operated on before 1927 and followed

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† Fellow in Internal Medicine.

TABLE 1. INCIDENCE OF RECURRENCE AS INDICATED IN THE LITERATURE

Authors	Time span	Total cases	Recurrence, % (all cases)
Cattell and Morgan, 1939 (1)	1928–37 2 yrs.	4,956 750	3.3 5.2*
Grace and Week, 1941 (7)	1930–39	245	6.1
Preston and Thompson, 1942 (4)	1930–39	294	12.0
Troell, 1944 (2)	1929–42	1,397	6.0
Hurxthal *et al.*, 1945 (3)	Prior to 1927, 10–20 yrs.	643	10.0 Persistence† 8.2 Recurrence
Bertelsen *et al.*, 1944 (8)	1937–44	647	4.9
VanderLaan and Swenson, 1947 (9)	1933–40	130	8.5
Goldman, 1948 (10)	1935–46	558	2.7
Ravdin *et al.*, 1949 (11)	1937–47	655	2.3
Crile, 1949 (5)			
Conservative surgery		*117*	*8.6*
Radical surgery		*144*	*2.1*

* Personal communication, H. J. Perkin.
† Primary hyperthyroidism.

for ten to twenty years, there was an incidence of 8.2 per cent. In this group there was also a 10 per cent incidence of persistence of symptoms. Since the period covered by his study was prior to 1927, it must be recognized that inadequate thyroidectomy was an important factor in the results. In 1942, Preston and Thompson (4) reported a series of 294 patients operated on between 1930 and 1939, with a recurrence rate of 13 per cent. In 1949 Crile (5) reported 261 cases with a recurrence rate in those who were treated conservatively in earlier years of 8.6 per cent, but in those having more radical surgery, an over-all incidence of 2.1 per cent. Cattell (6), in 1949, in a study of 381 patients of the first 1,000 patients prepared with antithyroid drugs and having subtotal thyroidectomy, who were followed for two years or more, found an incidence of 2.4 per cent.

Up to July 1949, 1,800 patients with hyperthyroidism, prepared with antithyroid drugs, have undergone radical subtotal thyroidectomy, and in this group 1,670 (93 per cent) have been carefully followed and the results evaluated (Fig. 1). One hundred and thirty patients were lost to follow-up and are not included in the study. This follow-up consisted of periodic basal metabolic check-up determinations and clinical evaluation every three months for the first year and annually for four subsequent years. All patients were examined either in the medical department of the

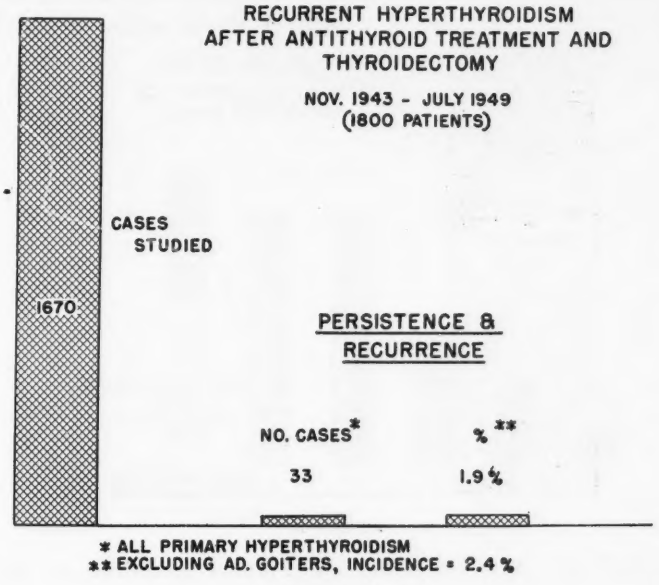

FIGURE 1

clinic or by their local physicians. Of the 1,670 patients, we found 33 with recurrent hyperthyroidism. All of those with recurrence had primary hyperthyroidism or diffuse toxic goiter. This gives an incidence of recurrent hyperthyroidism of 1.9 per cent. If cases of nodular goiter with hyperthyroidism (20 per cent of the total series) are excluded, the incidence of recurrent hyperthyroidism is 2.4 per cent in primary hyperthyroidism. There was no instance of recurrence of symptoms in nodular goiter with hyperthyroidism.

In the total series of 1,800 patients, 172 patients when initially seen were suffering from recurrent or persistent hyperthyroidism (Fig. 2). Following operative treatment of these 172 patients, 8 had further recurrence of the disease, an incidence of 4.7 per cent. These figures indicate that there

is twice the chance of development of hyperthyroidism when operation is performed for recurrent disease than when it is performed initially. The data should be of value in directing surgical therapy for the patient with recurrent hyperthyroidism, and would certainly justify the more radical excision of remnants.

CLINICAL FINDINGS IN 33 PATIENTS WITH RECURRENT HYPERTHYROIDISM

Of the 33 patients with recurrent hyperthyroidism, 25 were new patients previously treated at this clinic by antithyroid preparation and

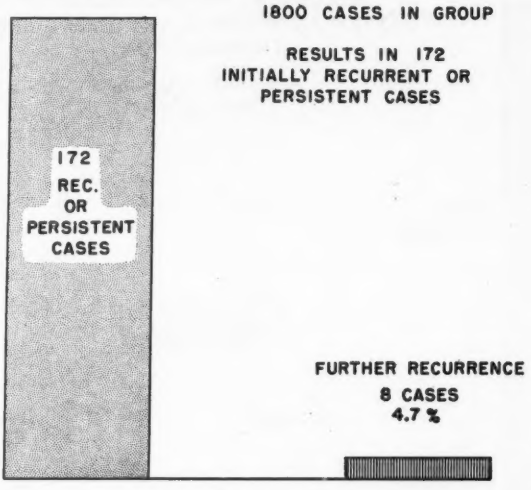

FIGURE 2

subtotal thyroidectomy, and 8 first came under treatment at the clinic because of recurrent or persistent hyperthyroidism. This introduces a confusing factor, yet it was necessary to include all cases with hyperthyroidism initially treated here with antithyroid drugs, whether they were new cases or cases of recurrent disease, since all patients were prepared with antithyroid drugs.

This group of 33 patients with recurrent hyperthyroidism has been analyzed relative to age, duration and severity of symptoms, time of onset and size of the gland. We have not attempted to determine the etiologic factors responsible for the development of the recurrent disease. There is, without question, one constant factor which seems to be indisputable and that has to do with the amount of the gland removed at the initial operation. This is the only factor which we have felt certain influenced the results. During the ten-year period of this study we have become more and more radical in the removal of the gland, so that at present, but

a few grams of tissue are left on each side when a radical subtotal thyroidectomy is performed. The technic that has consistently been employed has been that devised and described by Dr. Lahey, which includes wide exposure and dissection to make certain of the amount that is left, as well as to avoid postoperative complications. Our figures tend to support the fact that when antithyroid agents were initially used in the early part of the study there was considerable question as to how radical the operation should be, but it was soon apparent that an even more radical operation was essential since our initial rate of recurrence and persistence was higher than in the latter part of the study.

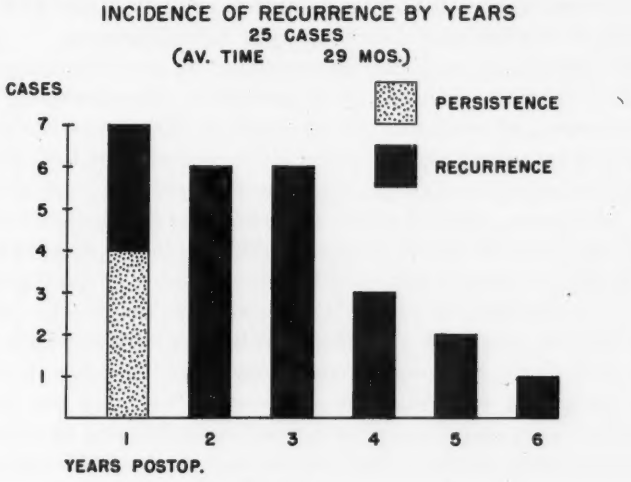

FIGURE 3

The average age of those patients in whom recurrence developed after the initial operative procedure was 37.3 years, yet the ages ranged from 11 to 69 years, indicating that recurrence can take place at any age. The age incidence follows the same decade distribution that is found in hyperthyroidism. The average age for patients who had a secondary operation for hyperthyroidism was 44.5 years and the ages ranged from 37 to 58 years.

In a consideration of the duration of symptoms in patients with a first recurrence it was found that the duration was only slightly increased over the average of all patients with hyperthyroidism; but in those with a second recurrence or persistence, it was considerably prolonged, averaging twenty-two months.

Although the duration of symptoms did not appear to be significant, the severity of the disease seemed to influence the incidence of recurrence,

since over 80 per cent of the patients who had recurrent disease were classified as having severe hyperthyroidism. This finding should be of value in the surgical approach to patients with severe hyperthyroidism; radical subtotal thyroidectomy should be initially considered in these cases.

The average time of onset of recurrence after operation was twenty-nine months. This figure does not include 4 patients who had persistence of the disease, and were operated on in the early period of this study. Our experience supports the view that if patients remain symptom-free for a period of five years following operation, only a rare instance of development of recurrence will be found subsequently (Fig. 3). This shows the rationale of the five-year follow-up after thyroidectomy.

In our experience with patients operated on for recurrence, the hyperthyroidism was not relieved by conservative procedures, including unilateral excision of remnants. Irrespective of the size of the remnants as determined by palpation, it is essential to perform a radical subtotal excision of remnants on both sides. The size of the gland, as well as the severity of the symptoms, seems to play a part in the development of recurrent hyperthyroidism. Of the 25 patients having an initial operation for hyperthyroidism, the average weight of the gland removed was 62 grams, whereas at the secondary operation, the average weight of the remnants removed was 22 grams. In view of the radical procedure which was carried out in this group it is certain that there was considerable regrowth of tissue. From our experience, it is necessary to carry out more radical removal of large glands, so that proportionately more tissue is removed. As a matter of practice, we plan to leave but 2 grams of tissue on each side in all cases, whether glands are small or large.

THE MANAGEMENT OF RECURRENT HYPERTHYROIDISM

Various methods were used in the management of the 33 patients in this study who had recurrent hyperthyroidism (Fig. 4). An attempt was made to control the symptoms of hyperthyroidism by iodine alone in 32 of the 33 patients. In 16 patients the attempt was successful, and these patients are still on constant iodine medication. Based upon this experience, it would seem that there is a chance of controlling recurrent hyperthyroidism by iodine alone in 50 per cent of the cases. Half of this group under satisfactory control had moderate-sized remnants and those with small remnants, although still taking iodine, appear to have a remission of the disease. Four other patients have been relieved by other nonoperative means: 1 patient had x-ray treatment, 1 had radioactive iodine and 2 are being maintained in an euthyroid state on Tapazole.

Thirteen of the 33 patients with recurrent hyperthyroidism were sub-

mitted to further surgery. Eight underwent operation because the disease was not controlled with iodine and the patients had large or increasing remnants. Two were under satisfactory control with iodine but they had large remnants, and it was considered that medical treatment could not be continued successfully. One patient had a small remnant but the hyperthyroidism could not be controlled with iodine. One patient had had previous recurrence of symptoms; operation was performed because the remnant was enlarging. The final patient who was operated on was not given a trial of iodine; operation was performed because of large remnants.

The treatment of this small series of patients who had recurrent hyper-

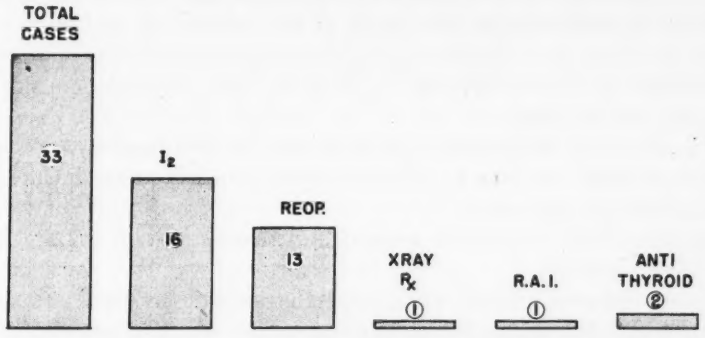

FIGURE 4

thyroidism does not reflect our total experience and method of treatment of patients with recurrent and persistent hyperthyroidism. During this same period of study, 172 patients were operated on, but an analysis of the results of their treatment is not included, since this experience will be reported later.

There were no deaths in this group of 33 patients and the postoperative complications following the initial procedure were negligible. In 1 patient malignant exophthalmos developed, 1 patient had a cerebrovascular accident, 1 patient had transient tetany and 1 patient had unilateral paralysis of the recurrent nerve. Following the secondary operation in the 13 cases reoperated on, 4 patients had myxedema, 2 had permanent tetany, 1 had transient tetany and 1 had unilateral paralysis of the recurrent nerve.

In a consideration of the best method of treatment for recurrent hyperthyroidism, the incidence of postoperative complications must be considered. If this is found to be high after the difficult operation of subtotal excision of remnants, obviously, some nonoperative means of control must be considered. We feel justified in continuing to advise subtotal

excision of remnants in all patients with recurrent or persistent hyperthyroidism who have large remnants, in view of the low rate of complications following operation in this clinic. Without question, all patients should first be given a trial of medical measures, including iodine and antithyroid drugs, and further experiences will probably show the advisability of using radioactive iodine in some of these problem cases.

SUMMARY

From 1943 to 1949, 1,800 patients with hyperthyroidism received antithyroid therapy followed by subtotal thyroidectomy. In this group, 1,670 cases, or 93 per cent, were followed for approximately three to eight years. Recurrent hyperthyroidism developed in 33 patients, an incidence of 1.9 per cent. In no patient operated on for nodular goiter with hyperthyroidism did recurrent symptoms develop and, if these cases are excluded, the incidence was 2.4 per cent.

The incidence of recurrence in patients who had recurrent hyperthyroidism when initially seen was 4.7 per cent, approximately twice the incidence following primary operation.

Hyperthyroidism was found to recur most commonly in the first three years after operation.

Of the 33 patients with recurrent hyperthyroidism, the disease was satisfactorily controlled by iodine medication in half of the group, in spite of moderate-sized remnants in some cases. In addition to this, the hyperthyroidism was controlled by antithyroid drugs in 2 cases, by x-ray therapy in 1 and by radioactive iodine in 1.

Thirteen patients were submitted to secondary operation because of failure of medical treatment. These patients were operated on without mortality and with a low rate of complications.

This study demonstrates that the use of antithyroid drugs has not materially altered the incidence of recurrent hyperthyroidism or the surgical approach to its treatment.

The most important factor in the avoidance of recurrent hyperthyroidism is the amount of thyroid tissue removed. To maintain a low incidence of recurrence, it is essential to do radical subtotal thyroidectomy.

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PHYSIOLOGY OF RECURRENT LARYNGEAL NERVE: REPORT ON PROGRESS*

JOHN A. MURTAGH, M.D. AND CLARENCE J. CAMPBELL, M.D.

From the Hitchcock Clinic and Dartmouth Medical School, Hanover, New Hampshire

AN ATTEMPT has been made to evaluate the fundamental background of recurrent laryngeal nerve paralysis by understanding better the details of normal laryngeal and recurrent larygneal nerve function (1, 2, 3). We have been interested in demonstrating, if possible, true laryngeal motor function, and in eliminating controversial opinion as to recurrent nerve innervation. Accordingly we developed an apparatus with which we could record photographically changes produced by the alteration of vocal cord position when the various nerves supplying the larynx were stimulated in experimental animals. We wanted to know why abductor function is lost first in the recurrent laryngeal nerve in the presence of a lesion or following trauma to the nerve; why the vocal cord remains in the mid line or near mid line following injury to the recurrent laryngeal nerve; whether there is motor function in the internal division of the superior laryngeal nerve (claimed by some authors) as an explanation for vocal cord position following recurrent laryngeal nerve trauma; what happens upon stimulation of the external division of the superior laryngeal nerve; and what responses are obtained when the recurrent laryngeal nerve and the superior laryngeal nerve are stimulated.

We found, as had been shown by previous workers, that stimulation of the recurrent laryngeal nerve produced adduction of the corresponding vocal cord; we could never produce predictable abduction. We found no evidence of reflex changes on stimulation of the central end of the cut recurrent laryngeal nerve. There was no evidence of motor function in the internal division of the superior laryngeal nerve on stimulation of the peripheral end of the sectioned nerve. We determined that the internal division carries afferent fibers involved in various reflexes affecting glottic motion and altering respiration.

Our findings revealed that the cricothyroid muscle has a strong adductor

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This article will be included in the bound volume of the 1952 "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1953.

function, that its function is not solely that of a tensor of the vocal cord, and that this muscle is responsible for maintaining the vocal cord in the mid line or near the mid line following injury to the recurrent laryngeal nerve (4, 5). When all other nerves to the larynx are sectioned, leaving only the external division of the superior laryngeal nerve intact, section of the external division or section of the vagus on that side produces abduction of the corresponding vocal cord. With these basic findings in mind, we went on with further studies of the recurrent laryngeal nerve itself, with the hope that we could determine the vulnerability of the abductor fibers and the reason for their early loss of function following any injury, or in the presence of any lesion involving the recurrent laryngeal nerve.

With quantitatively controlled stimuli of the nerves and muscles of the larynx, we found in general that the duration of stimulus necessary at comparable intensities was usually ten times greater for the muscle of abduction and the nerve supplying this muscle than it was for adduction. This led us to believe that, since usually nerves which are larger and more heavily medullated require stimuli of shorter duration, the nerve supply to adductor muscles would be from the larger, more heavily coated fibers. That this might be the case was further shown when it was attempted to differentiate between abductor and adductor nerve supply by means of chemical block. Using either diluted alcohol or cold, it was not possible to suppress adduction and demonstrate abduction, since the smaller fibers supplying the abductors were first affected by the blocking agents. In fact, we were never able, using controlled electrical stimuli of the recurrent laryngeal nerve, with or without blocking agents, to produce a predictable abduction. We were able to locate the branch to the posterior crico-arytenoid muscle, and any adequate stimulation of this nerve produced abduction. We did obtain abduction, but we never knew when to expect it; and the recurrence of abduction when stimulation of the recurrent nerve was accomplished was unexpected and unexplained.

Action potentials recorded from the recurrent laryngeal nerve show that the greatest activity occurs at the end of inspiration and at the beginning of expiration. This corresponds with the occurrence of the normal expiratory adduction of the vocal cords, and is taken to be a further indication of the preponderance of large fibers supplying adductors.

Up to this point it would appear that it has been shown that the larger nerve fibers making up the recurrent laryngeal nerve, supply adductor muscles; and judging from electrical records, there are more of these larger fibers than there are of the smaller, less heavily medullated fibers which supply abductor muscle and which are more sensitive to chemical blocking agents.

To get further evidence on fiber size and function, histologic studies, using osmic acid preparations, were made on all available recurrent laryngeal nerves of our experimental animals and on human nerves obtained at postmortem and at laryngectomy. These counts did not completely confirm the statement we have just made. If one considers that in general the small fibers are those less than 3 micra in diameter, actually they make up from 30 to 40 per cent of the normal nerve. However, counts in a case of recurrent laryngeal nerve palsy due to esophageal cancer bore out our contention by showing that the small fiber count was markedly diminished in the paralyzed nerve. We are now attempting to get experimental evidence on the degeneration that follows tying or applying tension or pressure to the recurrent laryngeal nerve of an animal *in situ*. Following the experimental procedure and using aseptic technique, the nerve is replaced in its bed, the wound is closed, and time for histologic degeneration allowed to elapse. Usually at the end of a period of about two weeks, the nerve is removed and histologic preparations are made of the nerve, both peripherally and centrally from the site of the experimental trauma. The number of experiments is not yet large, but results indicate that tying the nerve seems to produce little selective degeneration. At the end of two weeks approximately 50 per cent of the total number of fibers identifiable in the central end of the nerve are completely missing in the peripheral end; of the fibers remaining in the peripheral end and not degenerated, a slightly greater percentage seems to be present as smaller fibers. When a tension of 100 grams is applied for sixty seconds to the recurrent laryngeal nerve, there is produced a loss of about one third of the fibers and there exists a striking loss among the smaller fibers, though many of the remaining fibers of all sizes are degenerated. A tension of 150 grams produces an effect not dissimilar to that reported from ligation. About one half of the fibers disappear at the end of two weeks. Approximately all the remaining fibers show some degeneration. Obviously, we have been using too great tension. The only thing we can demonstrate at this time is that tension of over 100 grams produces serious degeneration.

These experiments are continuing, using less tension; and experiments are in progress on the effects of pressure on action potentials of the isolated recurrent laryngeal nerve freshly removed from the animal. In this type of experiment it should be possible to determine the changes produced in the different fiber-size groups by varying degrees of trauma.

SUMMARY

The recurrent laryngeal nerve is pre-eminently a motor nerve. There is no experimental evidence of reflex response to stimulation. The motor fibers of the nerve supply both adductors and the abductor of the vocal

cords. We have some evidence to show that the adductors are supplied by the larger fibers, and the abductor by the smaller fibers.

The work on experimental recurrent palsy is as yet incomplete, but there are definite indications that (a) ligation of the nerve produces degeneration or loss of function in large and small fibers in about equal proportion, and that (b) moderate tension (less than 150 grams for sixty seconds) produces degeneration and loss of function selectively in the smaller fibers.

There is no histologic or physiologic evidence that abductor fibers are in separate bundles in the recurrent laryngeal nerve.

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The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send FOUR COPIES of the title and abstract to the Vice-President, Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore 5, Maryland, not later than FEBRUARY 1, 1953. **It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:**

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three additional copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

The Endocrine Society

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953*.

FIFTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

Sponsored by

THE ENDOCRINE SOCIETY

Roney Plaza Hotel

Miami Beach, Florida *November 3–8, 1952*

The faculty will consist of 20 clinicians and investigators eminently qualified in the fields of endocrinology and metabolism.

The program will consist of lectures, clinics, laboratory demonstrations and scientific exhibits.

The 64 lectures to be presented will cover all phases of endocrinology and metabolic disorders. Ample time will be given for discussions and answers at the end of each session and registrants are encouraged to contact members of the faculty for individual discussions. The entire program has been designed to interest not only the specialist but those in general practice and has been approved by the American Academy of General Practice for postgraduate credit.

A simultaneous translation of all lectures into Spanish will be provided through earphones.

A copy of the proceedings of the Assembly will be available to all registrants.

The Roney Plaza with its tropical gardens, outdoor swimming pool, putting greens, private beach and Cabana Club, has long been recognized as one of Florida's great beach hotels and offers special convention rates to members of this Assembly. The Surf Room, in which the sessions will be held, is delightfully air conditioned and has subdued lighting which permits taking of notes and viewing lantern slides with comfort.

A fee of $75 will be charged for the entire course and the attendance will be limited to 200. *Registration will be in the order of checks received and will close on October 3, 1952.* Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Please forward application on your letterhead, together with check payable to The Endocrine Society, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 N. Walker Street, Oklahoma City 3, before October 3, 1952. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida, and the hotel advised that you are attending this Postgraduate Assembly.

[For program, *see* August issue of the Journal.]

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9, 1953*, in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953*. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.

RECIPIENTS OF VAN METER PRIZE AWARD FOR 1952

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The Van Meter Prize for 1952 was awarded to *Dr. Martin Sonenberg* for the essay on "Radioactive Thyrotropic Hormone Preparations," in collaboration with Drs. Albert S. Keston, William L. Money, and Rulon W. Rawson.

Dr. Sonenberg was born in New York City in 1920. He received his B.A. degree from the University of Pennsylvania in 1941 and was graduated from New York University College of Medicine in 1944. After an internship at the Beth Israel Hospital in New York and a medical residency on the Third (New York University) Division of Goldwater Memorial Hospital, Dr. Sonenberg spent two years in the Medical Corps of the United States Navy. He returned to the Department of Chemistry of New York University College of Medicine in 1948. Since 1949, he has been associated with the Sloan-Kettering Institute and Memorial Center for Cancer and

Allied Diseases, where he now is a Clinical Assistant in the Department of Medicine, an Assistant in the Sloan-Kettering Institute, and an Instructor in Medicine at Cornell University Medical School. Dr. Sonenberg was awarded the degree of Doctor of Philosophy in Biochemistry at New York University in 1952.

Dr. Keston was born in New York City in 1911. He received the degrees of A.B. and M.S. in Michigan in 1932, and the degree of Ph.D. from Yale in 1935. He was at the College of Physicians and Surgeons, Columbia University, 1937–1941; was Instructor in Chemistry, City College of New York, 1941–1944; Research Associate, College of Medicine, New York University, 1944–1947; and has been Assistant Professor at that institution since 1947.

Dr. Money was born in Centerville, Rhode Island, in 1914. He received the B.A. degree from Brown University in 1943 and the Ph.D. degree from Harvard University in 1947. He was a Teaching Fellow at Harvard, 1944–1945; Research Fellow in Medicine, Harvard, and Massachusetts General Hospital, 1947–1948; Assistant, Sloan-Kettering Institute, 1949–1950; Research Assistant, Cornell University Medical School, 1949; Research Associate in Medicine (Physiology), Cornell University Medical School, 1950; Associate, Sloan-Kettering Institute, 1951; and is now Assistant Professor of Biology, Sloan-Kettering Division, Cornell University Medical School, New York.

Dr. Rawson was born in Idaho Falls in 1908. He attended the University of Utah, received the degree of M.B. from Northwestern in 1937, and the M.D. degree in 1938. He was a research Fellow at Harvard, 1938–1941; at the Massachusetts General Hospital, 1941–1944; Associate in Medicine, at Harvard 1945–1947; Assistant Professor, 1946–1947; Associate Professor, Cornell University Medical School 1948; Chief, Department of Clinical Investigation, Sloan-Kettering Institute, 1948; Attending Physician, Memorial Hospital, New York, 1948; and has been Professor of Medicine, Sloan-Kettering Division, Cornell University Medical School, since 1948.

FIRST HONORABLE MENTION

First Honorable Mention was given to the essay by *Dr. Ray H. Rosenman*, with Dr. Sanford O. Byers and Dr. Meyer Friedman, entitled "The Mechanism Responsible for the Altered Blood Cholesterol Content in Deranged Thyroid States."

Dr. Rosenman was born on November 17, 1920. He attended the undergraduate and the medical school of the University of Michigan, where he received an M.D. degree. His internship was at the Michael Reese Hospital, Chicago, and his postgraduate training consisted of a residency in Pathology at the Wayne County General Hospital, and residencies in Internal Medicine and Cardiology at the Michael Reese Hospital, Chicago.

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He served in the U. S. Army Medical Corps from 1946 to 1948. In 1950 he was a Research Fellow of the American Heart Association. At present he is a member of the Department of Medicine, Mount Zion Hospital, San Francisco, Consultant in Medicine at the U.S. Public Health Service Hospital, San Francisco, and a Research Associate of the Harold Brunn Institute, Mount Zion Hospital.

Dr. Byers was born on May 1, 1918. His undergraduate training was pursued at the Rensselaer Polytechnic Institute. His postgraduate training was obtained at the University of Cincinnati where he received an M.A. degree in 1940 and a Ph.D. degree in 1944. He has been an Instructor in the Departments of Biochemistry and Physiology, Washington University, St. Louis, Research Biochemist of the National Drug Company, Philadelphia, and since 1947 he has been Chief Biochemist of the Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco.

Dr. Friedman was born on July 13, 1910. He attended Yale University 1927–1931 and the Johns Hopkins Medical School 1931–1935, where he received an M.D. degree. His postgraduate training consisted of an internship at the Kansas City General Hospital, and residencies in Internal Medicine and Cardiology at Michael Reese Hospital, Chicago, and at the University of Wisconsin General Hospital. He served in the U. S. Army Medical Corps from 1945 to 1948. At present he is Associate Chief of Medicine, Mount Zion Hospital, San Francisco, Director of the Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, Instructor in Medicine, Stanford Medical School and Consultant in Cardiology, Veterans Administration. He is a Fellow of the American Board of Internal Medicine and of the American Board of Cardiovascular Diseases.

SECOND HONORABLE MENTION

Second Honorable Mention was given to the essay by *Dr. Chalmers M. Gemmill*, entitled "Enzymatic Mechanisms of Thyroxine."

Dr. Gemmill was born in Cresson, Pa., on November 24, 1901. He attended William Penn Charter School in Philadelphia, 1914–1918; Lafayette College, Easton, Pa., 1918–1922 (B.S. degree); and Johns Hopkins University Medical School 1922–1926 (M.D. degree). He carried on graduate study at Johns Hopkins University, at the University of Chicago, at Heidelberg, Germany, at Cambridge, England, and at Lund, Sweden. He rose from Assistant to Associate Professor in the Department of Physiology, Johns Hopkins University Medical School, during the period 1926–1941; and from Lt. Comdr. to Captain, U. S. Navy, during the period 1941–1945. He has been Professor of Pharmacology, University of Virginia Medical School, since 1945.